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BACTERIOLOGIC STUDIES OF THE X-RADIATED DOG*

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Aureomycin, an antibiotic with a broad bacterial spectrum, has been used in the therapy of the radiation syndrome in dogs and has been found to delay the onset of morbidity and mortality. Clinical and pathologic observations of this study have been reported in the preceding paper.[1] The bacteriologic observations made in this study are in agreement with the reports of Chrom,[2] Lawrence and Tennant,[3] Bennett, Rekers, and Howland,[4] and Miller, Hammond, and Tompkins,[5] which indicate that the intestinal tract is an important source of infection in the x-radiated animal. Vincent[6] studied the flora of the small intestine of normal and radiated rats and found that the coliform and pathogenic staphylococci increased markedly following radiation. He suggested that there is a possible relationship between radiation damage to the small intestine and alteration of the flora of the intestinal tract.

This report concerns the bacteriologic studies made on a group of 24 dogs that received 450 r. of whole body x-radiation. Twelve of these animals were treated with aureomycin. The remainder served as controls. Since blood cultures obtained from x-radiated dogs show a preponderance of gram-negative bacilli,[4] it is important to study in detail the flora of the intestinal tract, which is the major potential reservoir of such organisms. In this study analysis of the feces showed an increase in the number of coliform bacteria and staphylococci in all animals post-radiation. The coliform bacteria, however, increased to an even greater extent in irradiated dogs treated with aureomycin. Blood culture studies following irradiation indicated that the number of positive cultures in the control dogs increased, while the number of positive cultures in the treated dogs decreased.

* This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N.Y.

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Methods

In this study 24 adult, healthy, mongrel dogs received 450 r. of whole body x-radiation. Immediately following irradiation one-half of the animals received aureomycin* at the rate of 100 mg. per kg. per 24 hours. This medication was continued every 6 hours for 28 days. Details of animal care and radiation factors were given in the preceding paper.[1]

During the course of the experiment venous blood was obtained from the external jugular vein after preparation of the skin by shaving and applying tincture of iodine. For aerobic culture, thioglycollate broth was inoculated with 2 cc. of blood, and after 24 hours of incubation at 37° C., a loopful of the broth was transferred to a blood agar plate. For anaerobic cultures, after 48 hours of incubation, 1 cc. of broth was pipetted from the bottom of the thioglycollate tube and transferred to anaerobic agar. These plates were then incubated in an anaerobic jar for 48 hours.

Cultures of heart's blood, liver, spleen, and lung for aerobic and anaerobic culture were obtained at necropsy. All necropsies were carried out within 4 hours after death, and in most instances, within the first hour.

Complete taxonomic studies with exact species and strain differentiation were not done on the bacteria isolated, so that a positive identification was made only as to genera.

A quantitative analysis of fecal flora was used to study coliform bacteria, staphylococci, and streptococci. A uniform suspension of fecal material was made by shaking a 0.5 gm. sample of fresh feces in 100 cc. of sterile saline solution in a mechanical shaker for 20 minutes. Pour plates, using various dilutions of this saline suspension, were made with mannitol salt agar for staphylococci identification and with EMB† media for the coliform organisms. For streptococci isolation the saline suspension was distributed on the surface of Mitis salivarius† media with a sterile glass spreader. The colonies on the plate were counted after 24 hours of incubation at 37° C. The feces were also plated on Shigella-Salmonella† media for the isolation of pathogens. No attempt was made in this study to differentiate between species. The daily fecal bacterial counts for each group of dogs were averaged and the log of this daily average is plotted in Text-figures 1 to 4.

The sensitivity to aureomycin of the various organisms isolated from

* Supplied by Lederle Laboratories Division, American Cyanamid Corporation, New York, N.Y.

† Difco Laboratories, Detroit, Mich.

blood cultures, necropsy material, and feces was determined by a tube dilution method, using serial dilutions of the antibiotic with a standard amount of a 24 hour broth culture of the organism. Readings were made at the end of 24 hours. The end point is defined in terms of the aureomycin content of the tube containing the least amount of aureomycin in which no growth has occurred. These readings, therefore, presumably represent the bactericidal level.

The above bacteriologic studies were made for a 2-week period prior to x-radiation and for a 4 weeks post-radiation period.

TABLE I
Percentage of Positive Blood Cultures

	Pre-radiation, aerobes and anaerobes	Post-radiation, aerobes and anaerobes
Treated group		
Number cultured	71	176
Positive cultures	8.5%	6.3%
Control group		
Number cultured	71	149
Positive cultures	1.4%	14.8%

RESULTS

The percentage of positive blood cultures in the control group increased following irradiation and exceeded the number of positive cultures in the treated group following irradiation. This is shown in Table I.

Of the positive cultures in both groups post-radiation, 22 per cent occurred during the first week, 6 per cent in the second week, 36 per cent in the third week, and 36 per cent in the fourth week. Of all the positive anaerobic cultures post-radiation, 12 showed gram-positive bacilli which were of the Clostridia group, 3 were gram-negative bacilli, and 11 were gram-positive cocci.

The incidence of positive blood cultures was no higher in the dogs that died. Blood cultures were obtained within the 24-hour period before death in 8 of the 12 dogs that died. Only 2 were positive. One from a control dog showed *Escherichia coli*, which was isolated also from the blood and tissues of the dog when it died 6 hours later. The other culture was taken from one of the treated dogs and was positive for Pseudomonas which was cultured also from the heart's blood at necropsy. Seven of the control and 5 of the treated dogs died post-radiation. Cultures obtained at necropsy from 6 of the control dogs

were positive for *E. coli*. *Aerobacter aerogenes* was recovered from the other control dog that died. A Pseudomonas organism was recovered from 2 of the treated dogs and Proteus from another treated dog that died. Necropsy cultures from 2 of the treated dogs were negative. Table II shows the types of aerobic bacteria found in blood and necropsy cultures following radiation.

TABLE II

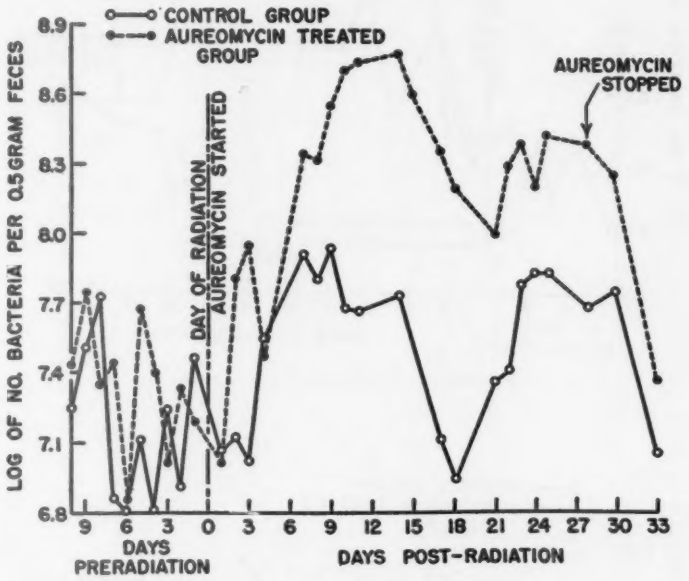
Aerobic Bacteria Found in Blood and Necropsy Material Following Radiation

Dog no.	Week post-radiation	Source	Organism	Sensitivity
1 Control	2	Blood	Non-hemolytic streptococcus	0.195
2 Control	2	Blood	*A. aerogenes*	
3 Control	2	Necropsy	*E. coli*	3.12
4 Control	2	Necropsy	*E. coli*	1.56
5 Control	3	Blood	*Staph. albus*	25.00
	3	Blood	Non-hemolytic streptococcus	0.195
6 Control	3	Necropsy	*A. aerogenes*	3.12
7 Control	3	Necropsy	*E. coli*	3.12
8 Control	3	Necropsy	*E. coli*	1.56
9 Control	3	Necropsy	*E. coli*	3.12
10 Control	3	Blood	Non-hemolytic streptococcus	0.195
	4	Blood	*E. coli*	
	4	Necropsy	*E. coli*	1.56
11 Control	4	Blood	Non-hemolytic streptococcus	
12 Control	4	Blood	*Strep. viridans*	12.5
13 Treated	2	Blood	Diphtheroids	
	3	Blood	Pseudomonas	12.5
	3	Necropsy	Pseudomonas	25.0
14 Treated	3	Blood	*Strep. viridans*	0.195
15 Treated	3	Blood	*Staph. albus*	0.195
16 Treated	4	Necropsy	Proteus	50.0
17 Treated	4	Necropsy	Pseudomonas	25.0

Examination of Feces

Text-figure 1 shows that the number of coliform organisms in the feces increased markedly in both the control and treated groups following irradiation, reaching maximum levels during the second week. The coliform bacteria increased to a much greater extent in the aureomycin treated dogs, and remained at a high level until therapy was discontinued, after which the count dropped abruptly to the pre-radiation value. The decrease in the counts from the 15th to the 25th days may be accounted for in part by the anorexia which occurred in both groups during this period. Text-figures 2 and 3 show that similar increases occurred in the staphylococcus and streptococcus counts in the treated animals and in the staphylococcus count in the controls. In

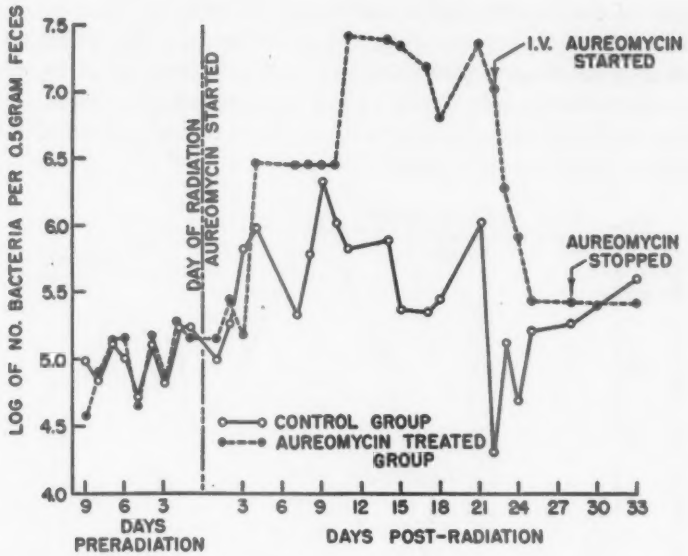
certain of the treated dogs a sudden drop in staphylococci coincided with the start of intravenous aureomycin therapy. The incidence of Proteus in the treated group also increased post-radiation. No organisms ordinarily considered to be pathogenic were isolated. The increases in fecal bacterial counts occurred during the period when the leukocyte counts of the blood were lowest.



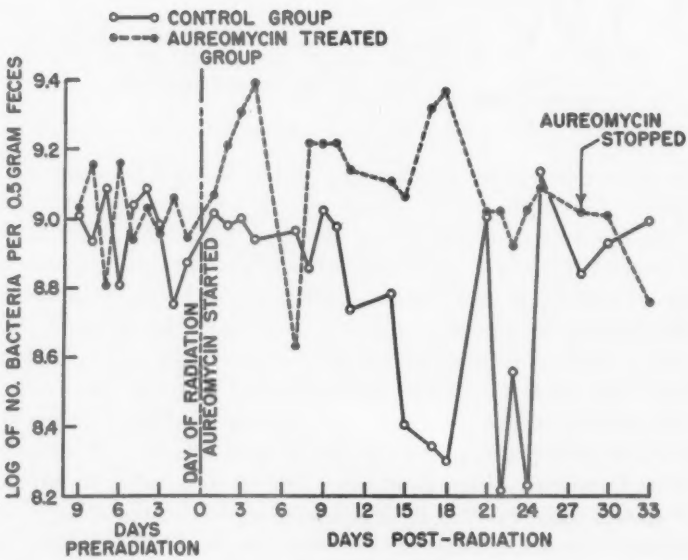
Text-figure 1. Fecal coliform bacterial count.

In order to determine whether this shift in bowel flora was primarily an effect of irradiation or of aureomycin, to a group of 4 normal dogs aureomycin was administered in identical dosage for 4 weeks. As shown in Text-figure 4, the counts of coliform bacteria and staphylococci showed increases during the 3rd week of aureomycin administration similar to, but less marked than, those demonstrated by the irradiated treated animals from the 10th to the 15th days post-radiation. There was essentially no change in the count of streptococci.

The results of the sensitivity tests of the bacteria isolated from blood and necropsy cultures are shown in Table II. All organisms isolated at necropsy from the control dogs were sensitive to aureomycin whereas those isolated at necropsy from the treated animals were resistant. The sensitivities of the organisms isolated from the feces before, and at weekly intervals after, irradiation are shown in Tables III and IV. The sensitivity of the staphylococci remained essentially

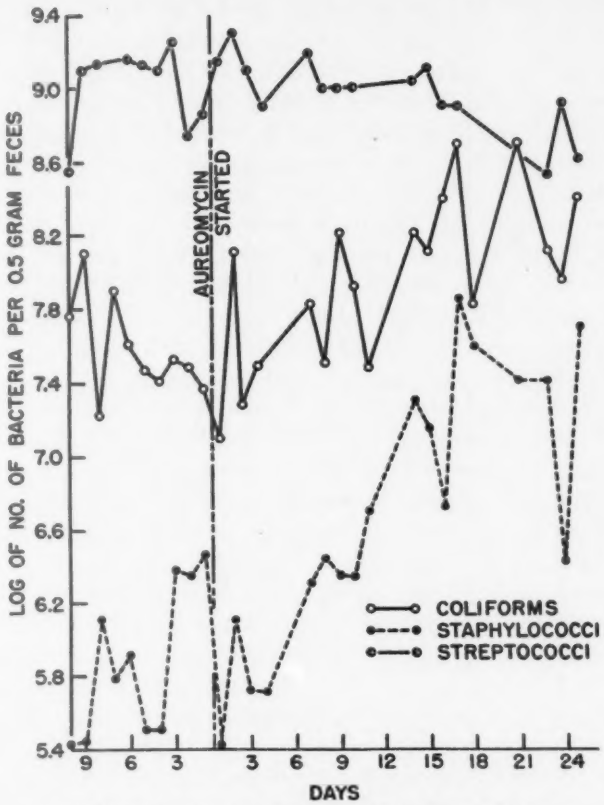


Text-figure 2. Fecal staphylococcus count.



Text-figure 3. Fecal streptococcus count.

unchanged in both groups, whereas the sensitivity of the streptococci decreased in both groups following irradiation. All Proteus strains were resistant to aureomycin before and after irradiation. The sensi-



Text-figure 4. Fecal bacterial counts in non-radiated dogs.

tivity to aureomycin of the coliform organisms from the treated dogs decreased markedly after irradiation but remained unchanged in the untreated group.

Discussion

The delayed onset of morbidity and mortality in the aureomycin treated dog that has received a large dose of whole body x-radiation supports the concept that infection plays an important rôle in the radiation syndrome. The view that the bacterial flora of the bowel is an important source of infection to the x-radiated animal is substantiated by the isolation from necropsy material of bacteria which are

TABLE III

In Vitro Aureomycin Sensitivities in μg. per cc.

	Dog no.	Fecal coliforms				Fecal Proteus			
		Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin	Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin
Controls	1516	1.56	3.12	3.12	1.56			100	
	1548	3.12	1.56			50			
	1552	25.0	12.5	3.12	3.12		50	100	
	1553	3.12	1.56	3.12		50			
	1559	1.56	3.12	3.12	3.12	100	3.12		
Average		7.0	4.0	3.0	3.0	66	27	100	
Treated	1554	1.56	100	12.5	50	100	100+	100	100
	1557	3.2	100	12.5	25	100+	100	50	100+
	1558	3.12	100	12.5		100+	100+	50	
	1565	1.56	50	6.25	50	100	100	100	50
	1567	3.12	50	12.5	12.5	100+	100	100	25
Average		2.5	80	11	35	100+	100+	80	70

the normal inhabitants of the intestinal tract. These types of bacteria were recovered also 6 to 12 hours pre-mortem in blood cultures from 2 of the dogs. Clostridia, which are also normally found in the bowel flora, were recovered in a large number of the anaerobic pre-mortem blood cultures. It is believed by some workers that a positive blood culture can be obtained uniformly from animals in the agonal state. However, blood cultures obtained a few hours prior to death and necropsy cultures from many of the treated dogs have been negative. All organisms isolated from necropsy material of the control dogs were sensitive to aureomycin. This may indicate that sepsis might have been prevented had these dogs received effective antibiotic therapy. All organisms isolated from the treated dogs at necropsy were resistant to high concentrations of aureomycin.

Basic factors contributing to the spread of enteric infection to other parts of the body of the radiated animal are damaged capillaries and ulcerations of the intestinal mucosa, damage of the reticulo-endothelial barriers, destruction of mesenteric lymph nodes, and leukopenia. In guinea-pigs which have been infected with the cholera vibrio, Burrows, Deupree, and Moore[7] reported a marked fall in coproantibody titer in the feces coincident with the invasion of other body tissues by the vibrio following irradiation. They stated that if natural immunity to intestinal bacteria is related to coproantibody, then the inhibition of this immune mechanism in the irradiated animal may account for a bacteremia of intestinal origin. Studies to determine the rôle of coproantibody and its correlation with fecal bacterial counts in the dog are now being made in this laboratory.

The increased number of coliform bacteria and staphylococci in the bowel of these radiated dogs may be of significance in the radiation syndrome. Alterations in environmental conditions in the intestinal tract may be responsible in part for the increase of the bacteria in the bowel. It has been shown that the pH of gastric juice is increased following irradiation.[8] There may also be qualitative and/or quantitative changes in the intestinal and pancreatic secretions.

The greater increase in fecal bacterial counts in the treated dogs than in the control dogs is of considerable interest. Dearing and Heilman[9] reported that coliform and gram-positive organisms are practically eliminated from the feces of humans who have received a therapeutic dose of aureomycin for from 1 to 3 days. In this laboratory comparatively long-term administration of aureomycin to normal dogs is followed by an increase in the number of coliforms and staphylococci in the feces. This increase is detected after 7 days of

TABLE IV

In Vitro Aureomycin Sensitivities in μg. per cc.

	Dog no.	Fecal staphylococci				Fecal streptococci			
		Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin	Pre-treatment	7 days after starting aureomycin	14 days after starting aureomycin	21 days after starting aureomycin
Controls	1516	12.5		12.5	12.5	0.195	0.195	25	12.5
	1548	25	12.5			0.195	12.5		
	1552	25	12.5	25	12.5	25		12.5	12.5
	1553	0.195		12.5		25	12.5		
	1559	0.195	12.5	25	12.5	0.195	1.56	0.195	12.5
Average		13	12.5	19	12.5	10	5	12.5	12.5
Treated	1554	50			12.5		12.5	25	25
	1557	12.5			12.5	3.12	12.5	12.5	12.5
	1558	25	12.5			12.5		25	
	1565	0.195	12.5	12.5	12.5	0.195	3.12	25	12.5
	1567	0.195			12.5	25	0.195	12.5	12.5
Average		18	12.5	12.5	12.5	8	7	20	15

oral aureomycin administration. These results are supported by the work of Marshall, Palmer, and Kirsner,[10] who found that, after administration of aureomycin, an initial decrease in fecal bacterial count occurs, followed by a rise of varying degree with *E. coli* becoming the predominant organism.

The secondary increase in the fecal coliform count may be partially explained with reference to the sensitivity tests, which showed that the coliform organisms from irradiated-treated and normal-treated dogs became resistant to aureomycin after 7 days of administration. It is also possible that aureomycin-dependent organisms may develop. A growth-promoting effect of aureomycin on these bacteria must also be considered, although initial experiments in this laboratory do not lend evidence to support this concept.[11] The increase in bacterial flora in irradiated dogs may follow the decrease in coproantibody titer reported by Burrows *et al.*,[7] since the withdrawal of this defense mechanism could allow bacteria in the bowel to multiply unchecked. It may be possible that a bacteriophage, normally present in the intestinal contents, is either inhibited or destroyed by x-radiation and/or by aureomycin.

It was found that following intravenous administration of aureomycin there was a sudden decrease in the fecal staphylococcus count. This may indicate that a bactericidal level of aureomycin was attained in the bowel following excretion of high concentrations of the antibiotic in the bile.[12]

It is of interest to note that the changes in fecal flora were somewhat different in a separate group of dogs that received oral aureomycin, streptomycin, and penicillin, in clinical dosage range, simultaneously.[11] The coliform bacteria in the treated animals again showed a marked increase following radiation. However, the gram-positive organisms, presumably as a result of penicillin therapy, remained at the pre-radiation level.

The deaths observed in both groups of animals were in most instances related to hemorrhagic tendencies present in various parts of the body. The mechanism by which this hemorrhage is produced is undoubtedly related to derangements in the vascular system and in the coagulation mechanisms. Oral administration of aureomycin decreases the extent of the ulceration and hemorrhage in the bowel wall. However, the hemorrhagic tendencies elsewhere are not altered. Certain of the toxic products of enteric organisms have been shown to cause alteration in vascular integrity.[13] If the increase in coliform organisms in the bowel causes an overproduction of lytic toxins which in turn

may cause hemorrhage, one might anticipate an increased tendency to local bleeding within the bowel in the treated animals. This did not occur. Even with the greater increase of fecal organisms as the result of aureomycin therapy, the absence of a hemorrhagic tendency within the bowel in the treated dogs would point toward the hemorrhage as a direct effect of the irradiation on the vascular walls and coagulation mechanism rather than a secondary effect of toxins elaborated in the lumen of the bowel from these organisms and circulated elsewhere.

This hemorrhagic diathesis contributes directly to the mortality from x-radiation. However, it can be reasoned from the evidence presented here that bacterial infection also must contribute to the morbidity and mortality of the radiation syndrome and in certain of the animals may have been the major cause of death, particularly in early stages.

Summary

Twenty-four adult dogs received 450 r. of total body x-radiation. Of these dogs, 12 received aureomycin at the rate of 100 mg. per kg. per 24 hours, for 28 days following radiation. Twelve animals exposed simultaneously served as controls. Bacteriologic studies of blood, necropsy material, and feces of radiated and normal dogs were made.

The incidence of positive blood cultures post-radiation was greater in the control group than in the treated group.

The fecal coliform bacteria, staphylococci, and streptococci increased to a greater extent post-radiation in the treated than in the control group.

Aureomycin resistant organisms were obtained from necropsy cultures from the treated dogs. The bacteria isolated from the control dogs were sensitive to aureomycin. The sensitivity to aureomycin of the fecal coliform bacteria and streptococci decreased post-radiation in the treated dogs. Bacterial strains resistant to high concentrations of aureomycin developed.

REFERENCES

1. Furth, F. W., Coulter, M. P., and Howland, J. W. The effect of aureomycin on the radiation syndrome in dogs. *Am. J. Path.*, 1952, **28**, 25–36.

2. Chrom, S. A. Studies on the effect of roentgen rays upon the intestinal epithelium and upon the reticulo-endothelial cells of the liver and spleen. *Acta radiol.*, 1935, **16**, 641–660.

3. Lawrence, J. H., and Tennant, R. The comparative effects of neutrons and x-rays on the whole body. *J. Exper. Med.*, 1937, **66**, 667–688.

4. Bennett, L. R., Rekers, P. E., and Howland, J. W. Influence of infection on the hematological effects and mortality following mid-lethal roentgen irradiation. *Radiology*, 1951, **57**, 99–103.

5. Miller, C. P., Hammond, C. W., and Tompkins, M. The incidence of bacteremia in mice subjected to total body x-radiation. *Science*, 1950, 111, 540–541.

6. Vincent, J. G. Bacteriological aspects of a toxic factor produced by irradiation damage to the intestinal tract. UCLA–15, Quarterly Progress Report (Classified), 1949, 50–56.

7. Burrows, W., Deupree, N. G., and Moore, D. E. Fecal and urinary response to cholera vaccine in x-irradiated guinea pigs. Argonne National Laboratory, Quarterly Report, ANL–4451, 1950, p. 109.

8. Prosser, C. L., Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., and Swift, M. N. The clinical sequence of physiological effects of ionizing radiation in animals. *Radiology*, 1947, 49, 299–313.

9. Dearing, W. H., and Heilman, F. R. The effect of aureomycin on the bacterial flora of the intestinal tract of man: a contribution to preoperative preparation. *Proc. Staff Meet., Mayo Clin.*, 1950, 25, 87–102.

10. Marshall, H. C., Jr., Palmer, W. L., and Kirsner, J. B. Effects of chemotherapeutic agents on fecal bacteria in patients with chronic ulcerative colitis. *J. A. M. A.*, 1950, 144, 900–903.

11. Unpublished data in this laboratory.

12. Herrell, W. E., and Heilman, F. R. Aureomycin: studies on absorption, diffusion and excretion. *Proc. Staff Meet., Mayo Clin.*, 1949, 24, 157–166.

13. Morgan, H. R. Pathologic changes produced in rabbits by a toxic somatic antigen derived from *Eberthella typhosa*. *Am. J. Path.*, 1943, 19, 135–145.

THE EFFECT OF AUREOMYCIN AND TERRAMYCIN ON THE X-RADIATED RAT*

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The administration of a large dose of whole body x-radiation to the rat produces a rather characteristic and uniform response. The syndrome which follows this irradiation is characterized by a profuse watery diarrhea, anorexia, apathy, and an unkempt appearance. These signs are usually evident by the third or fourth day post-radiation and persist for from 4 to 6 days. This syndrome differs somewhat from that seen in larger animals and in man. In the rat the principal early effect of irradiation is upon the gastro-intestinal tract. Cutaneous bleeding as seen in the guinea-pig and dog does not occur, although bleeding from the gastro-intestinal mucosa is seen frequently. Even though the radiation syndrome in the rat differs from that in larger animals, the rat can be used in screening tests of various agents which may be effective in the treatment of the radiation syndrome, since large numbers can be employed in individual experiments, and the diarrhea provides an easily observed objective sign for evaluating therapy.

Vincent[1] has reported a change in the bacterial flora of the small intestine of x-radiated rats, which he suggests may play a significant rôle in the radiation syndrome in these animals. He has found that the pathogenic staphylococci and coliform organisms in the small intestine increase following x-radiation. Miller, Hammond, and Tompkins[2] reported a high incidence of positive blood and splenic cultures in mice that had received 450 r. or 600 r. of x-radiation. The greatest incidence of bacteremia was observed on the day when the greatest mortality occurred. They treated x-radiated mice with various antibiotics and reported a reduction in mortality. These reports would seem to indicate that bacterial infection may play an important rôle in the morbidity and mortality of the acute radiation syndrome in the rat, and that antibiotics with a wide anti-bacterial spectrum would be effective in the treatment of this syndrome.

In a previous report[3] from this laboratory, preliminary results of antibiotic therapy in x-radiated rats and dogs were reported. Since then, studies on the effect of antibiotics on the radiation syndrome in

* This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N.Y.

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the rat have been continued and expanded. The study reported here includes observations on 119 x-radiated rats treated with aureomycin orally, and 75 treated with oral terramycin. An equal number of untreated x-radiated rats served as controls. The most obvious effect of these antibiotics is in decreasing the incidence of diarrhea post-radiation. Although aureomycin had no consistent effect on decreasing total mortality, terramycin altered the rate of mortality and decreased the 28 day mortality as well.

Methods

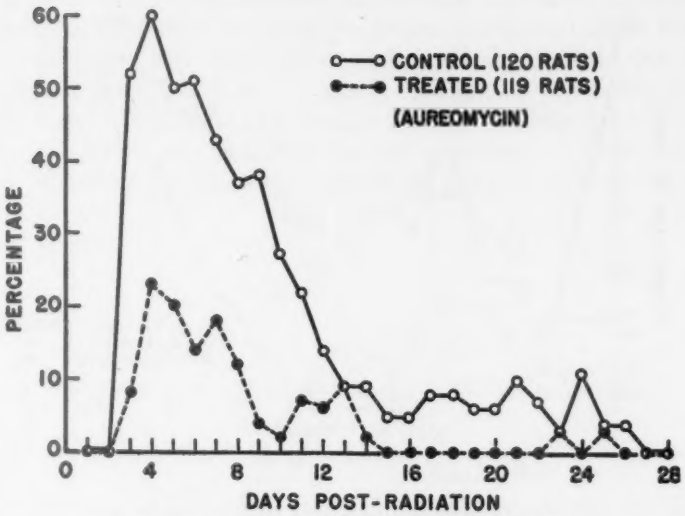
A total of 314 adult, male and female, Wistar rats weighing 150 to 180 gm. were used in this experiment. Each rat was housed in a separate cage to minimize cross-infection, and to facilitate the measurement of the amount of food eaten. Each rat was weighed, observed for diarrhea, and offered 15 gm. of high roughage food daily. Following irradiation, the treated animals received feed with which the antibiotic had been mixed. The concentration of aureomycin and terramycin was varied between 1 and 4 mg. per gm. of feed. During the first 5 days after irradiation, when anorexia was greatest, the feed with the highest concentration of drug was offered in an attempt to make the daily antibiotic dosage more uniform. By measuring the food eaten, it was found that the average 24-hour dose of aureomycin and terramycin was between 80 and 100 mg. per kg. None of the rats was given antibiotics previous to irradiation. The feed containing the drug was placed in the cage immediately after irradiation and was offered for a 28-day period post-radiation.

Radiation was administered with a 250 kv. Picker x-ray machine at 15 ma. using a parabolic aluminum filter with 0.5 mm. of copper. The target skin distance was 40 inches and the total target skin dose was 700 r. administered at the rate of 16 r. per min. Radiation was administered to groups of rats which contained equal numbers of experimental and control animals.

Results

The most striking effect of the administration of aureomycin and terramycin to the x-radiated rat was the decrease in the incidence of diarrhea. As shown in Text-figures 1 and 2, not over 23 per cent of the rats in the treated groups had diarrhea on any day post-radiation, in contrast to 60 per cent of the control rats. The treated animals appeared more lively than the controls, and did not have their unkempt appearance.

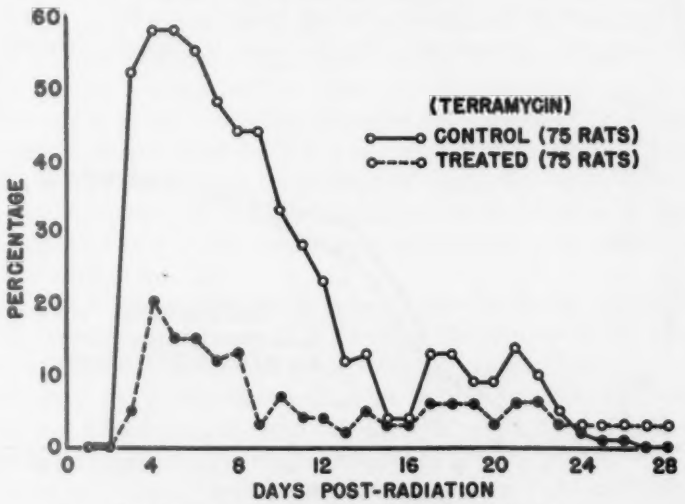
During the first 4 days after radiation an equal weight loss occurred



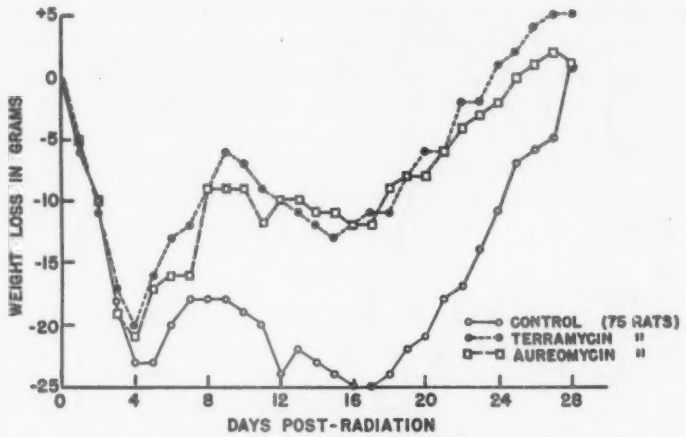
Text-figure 1. Percentage of living rats with diarrhea.

in all groups as shown in Text-figure 3. After this period the surviving antibiotic-treated rats began to regain weight at a much more rapid rate than the control animals, and continued to do so during the period of observation. There was no significant difference between the terramycin-treated and aureomycin-treated groups in this respect.

On the fifth day post-radiation, during the period of maximum



Text-figure 2. Percentage of living rats with diarrhea.



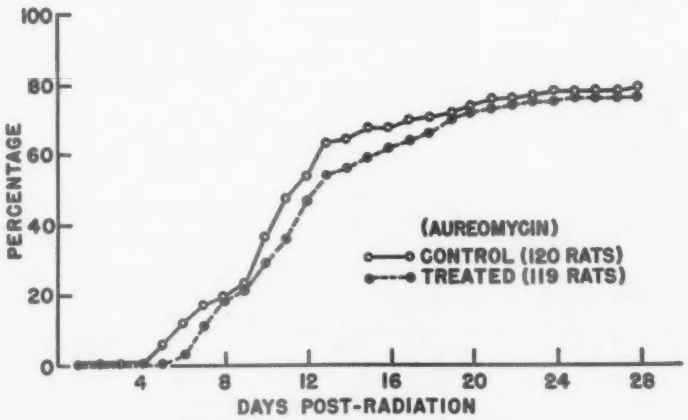
Text-figure 3. Average weight loss of living rats.

diarrhea and weight loss, the first rats died. This is shown in Text-figures 4 and 5. The rate at which the animals in the aureomycin-treated group died paralleled the mortality rate of the control group with no significant difference in the 28-day mortality. The mortality rate and 28-day mortality of the terramycin-treated group appeared to be significantly lower than that of the controls.

During the course of the experiment no obvious toxic effect of either antibiotic was observed.

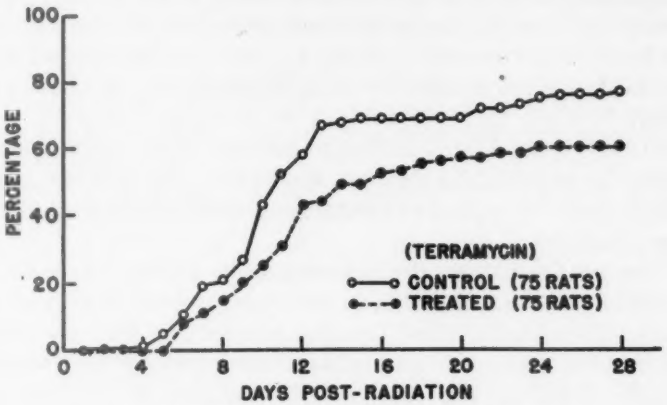
Discussion

The etiology of the profuse diarrhea which occurs in the rat following a large dose of x-radiation remains obscure. Conard[4] reported that



Text-figure 4. Percentage of mortality.

the tone of isolated rat intestine will increase during the period of exposure to x-radiation. However, he used a dose of irradiation which was greater than that used in these studies. Bloom[5] reported that the intestinal mucosa of the rat is severely damaged by 300 to 600 r. of whole body x-radiation. This damage is evident within a few hours after irradiation, with abnormal changes persisting for from 2 to 3 days. Histologically, the mucosal cells of the small intestine are normal



Text-figure 5. Percentage of mortality.

by the fifth day post-radiation. The time when mucosal damage occurs does not correlate well with the time when diarrhea is present. The changes in the bacterial flora of the intestinal tract, which Vincent[1] described, could play a rôle in causing the diarrhea. Unpublished data from this laboratory show that changes in the number of fecal coliform organisms and streptococci do occur post-radiation, and that aureomycin and terramycin probably decrease the number of these organisms in the feces during the first 3 to 5 days post-radiation. Changes in pH and enzymes within the bowel may occur secondarily to x-radiation and may also influence the motility of the bowel by inhibiting or altering the digestion and absorption of food material, thereby increasing the residual food bulk.

The effect of the antibiotics in reducing the diarrhea is more difficult to explain. In our laboratory it has been demonstrated that several antibiotics and sulfonamides aside from aureomycin and terramycin can reduce the post-radiation diarrhea in the rat. Because such a variety of substances can cause this effect, it seems inconceivable that they do so by direct effect on the autonomic nervous system. The one common denominator of all these substances is their antibacterial

potential. Therefore, it would seem most likely that their effect is through alteration in the bacterial environment of the intestinal tract.

Another interesting effect of the administration of these two antibiotics is the apparent stimulation of growth which they cause. This growth-stimulating effect has been noted with aureomycin in normal chicks and swine. It may be related to the "animal protein factor" described by Jukes *et al.*[6]

The mechanism of this growth stimulation is not apparent at the present time. Part of the regain of weight observed in this experiment may be due to the decrease in diarrhea. However, the increased weight gain, in the treated animals surviving, persisted after all diarrhea had ceased.

Aureomycin administration had no apparent effect on the mortality, despite the fact that diarrhea was diminished. The decrease in mortality in the group treated with terramycin, although not great, appears to be significant.

Other factors undoubtedly influence the mortality associated with x-radiation in the rat. It has been observed that many of the rats show considerable gastrointestinal bleeding between the fifth and twelfth days post-radiation, and that antibiotic administration does not influence the bleeding. The route of administration of the antibiotics directly affects the mortality. Any parenteral mode of administration markedly increases it.[7] Increasing the dosage of aureomycin during the period of anorexia and diarrhea by mixing it with the drinking water increases the mortality even though the dose does not exceed the toxic oral dose reported for the normal rat. It may be reasonable to assume that because of the possible increased permeability of the damaged intestinal epithelium, a greater proportion of the aureomycin is absorbed so that toxic levels of the drug in the tissues may be exceeded.

From the experiments reported here it is evident that although aureomycin and terramycin in therapeutic amounts can markedly decrease the diarrhea of x-radiated rats, these drugs do not appreciably influence mortality. Since these drugs are potent antibiotics with a wide antibacterial spectrum, it may be that bacterial infection does not play a major rôle in the mortality associated with large doses of x-radiation in the rat. Other more fundamental derangements in body function must occur which are major enough to be incompatible with life. The exact nature of these changes, which are certainly complex and interrelated, remains to be defined.

Summary

A group of 314 rats received 700 r. of total body x-radiation. Of these, 120 served as controls, 119 received aureomycin orally post-radiation, and 75 received terramycin orally post-radiation.

Aureomycin and terramycin lowered the incidence of post-radiation diarrhea. Terramycin delayed the onset of mortality and appeared to be effective in reducing mortality.

Rats which received either aureomycin or terramycin post-radiation regained weight more rapidly than did the controls.

REFERENCES

1. Vincent, J. G. Bacteriological aspects of a toxic factor produced by irradiation damage to the intestinal tract. UCLA–15, Quarterly Progress Report (Classified), 1949, 50–56.
2. Miller, C. P., Hammond, C. W., and Tompkins, M. The incidence of bacteremia in mice subjected to total body x-radiation. *Science*, 1950, **111**, 540–541.
3. Howland, J. W., Furth, F., Bennett, L. R., Coulter, M., and McDonnel, G. M. UR–94, the University of Rochester, 1949, 3–25.
4. Conard, R. A. Effect of x-irradiation on intestinal motility. *Federation Proc.*, 1950, **9**, p. 24.
5. Bloom, W. Histopathology of Irradiation from External and Internal Sources. National Nuclear Energy Series, McGraw Hill, 1948, **22**, Pt. 1, p. 508.
6. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B. Growth promoting effect of aureomycin on pigs. *Arch. Biochem.*, 1950, **26**, 324–325.
7. Unpublished data in this laboratory.

PULMONARY HEMORRHAGE
EXPERIMENTAL STUDIES*

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A review of necropsy material at the Children's Hospital has disclosed several factors which may be important in the pathogenesis of pulmonary hemorrhage in infants.[1] First, massive hemorrhage is much more common in newborn than in older infants. Second, congestion and its causes are fundamentally important. Finally, infection is basic as a single etiologic factor. To shed further light on the problem of pulmonary hemorrhage in infants, an experimental study was undertaken in which the following problems were considered.

1. Can aspiration of blood be differentiated from alveolar hemorrhage?

2. At what site does hemorrhage occur when the pulmonary circulation is overloaded with blood? Is the site of hemorrhage dependent on the direction of blood flow and on aeration of the lungs?

3. How important are the following factors in the development of pulmonary hemorrhage in the living adult rat?

   a. Pulmonary edema produced by alpha-naphthylthiourea (ANTU)
   b. Anticoagulants, heparin and dicumarol
   c. Increased blood volume
   d. Vigorous breathing produced by breathing against pressure and by narrowing of the trachea
   e. Anoxia

4. Can pulmonary hemorrhage be produced in the kitten by intratracheal injection of *Escherichia coli, Staphylococcus aureus,* gastric juice, and Baker's cat virus?

Adult rats, cats, and newborn kittens were used in the experimental studies. Methods will be described for each series. The trachea was tied before the thorax was opened; the lungs were removed *in toto* and fixed in a 10 per cent solution of formalin or in Klotz' solution. Blocks were taken after 48 hours and paraffin sections were stained with hematoxylin and eosin.

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Morphology of Pulmonary Hemorrhages

Traumatic Pulmonary Hemorrhage

Method. Traumatic pulmonary hemorrhage was produced in 7 adult rats by puncturing the lungs with needles inserted through various portions of the thorax. ANTU was given to one rat 5 hours before puncture. The animals were sacrificed immediately, or 24 hours later, by intracerebral injection of alcohol. Death occurred in a few seconds.

Results. A small area of hemorrhage was present about the needle-puncture mark. Alveolar hemorrhage and atelectasis were observed microscopically in this area. At one point an intrapulmonary vein had been entered. The vein appeared to be collapsed and surrounding hemorrhage was not extensive. After 24 hours, hemoglobin crystals were present in the area of hemorrhage. Hemorrhage appeared a little larger in the rat given ANTU.

Aspiration of Blood

Method. Ten adult rats were anesthetized with ether. Fresh chicken's blood was injected into the trachea with the animal in the supine position. One animal was placed in the prone position while a suspension of graphite in acacia was injected intratracheally. This animal died in 5 minutes. In 3 rats the pulmonary circulation was injected with human blood after aspiration, while the animals were dying.

Results. In rats receiving chicken's blood only, intratracheally in the supine position, gross examination of the posterior portions of all lobes showed lobular distribution of aspirated blood (Fig. 1). The rat receiving graphite in acacia, in the prone position, showed lobular distribution of the aspirated suspension in the anterior portions of the lungs. Microscopically, nucleated red cells of chicken's blood filled some collapsed alveoli. Entrapped air bubbles were seen frequently in dilated alveoli. In animals made to aspirate chicken's blood and then given large quantities (10 cc.) of human blood by pulmonary artery or vein, alveolar hemorrhages were composed of nucleated (aspirated) and non-nucleated (from the circulation) red blood cells. In some cases these were freely mixed; in others they were layered.

Overloading the Pulmonary Circulation of the Rat with Blood

Method. Adult rats were anesthetized with ether and immobilized in the supine position. A tracheal cannula was inserted. While the animal was dying, amounts of blood varying from 2 to 20 cc. were injected into various sites: (1) the pulmonary artery (with the aorta

tied), (2) pulmonary vein to a single lobe, or (3) the left ventricle (with aorta and pulmonary artery tied).

Results. Grossly, similar massive hemorrhages appeared regardless of the route of injection. During injection, hemorrhage began at focal points and then spread in all directions until the lung was dark red and firm. Pink foam appeared in the tracheal cannula during injection and the size of the lung increased in proportion to the amount of blood injected. Injections were stopped at various stages of hemorrhage. When injection was made into the left ventricle, more blood was required to produce massive hemorrhage than when injection was made into the pulmonary artery. When both lungs were collapsed, more blood was required to produce massive hemorrhage than when the lungs were expanded. With part of one lung collapsed, hemorrhage occurred first in the collapsed area and the immediately surrounding tissues. When an area of consolidation was present, hemorrhage occurred first around this area. The amount of blood needed to produce hemorrhage was the same for rats in which the lung had been made edematous by ANTU[2] as for rats which had not been given ANTU. Microscopically, in the expanded lung, capillary hemorrhage appeared first, then perivenous hemorrhage and later periarterial hemorrhage, as greater amounts of blood were injected. To differentiate between arteries and veins in the lung of the rat, a suspension of lycopodium[3] was injected into the pulmonary artery (in 2 rats) or pulmonary vein (in 2 rats) and the structural differences of intrapulmonary arteries and veins were studied in paraffin slides stained with hematoxylin and eosin. When the lungs were collapsed, perivenous hemorrhages were more extensive than when the lungs were expanded (Fig. 2). Rupture of the venous wall was observed occasionally, especially at branching points. Some perivascular hemorrhages appeared to be due to spread of capillary hemorrhage about the vessel. About areas of consolidation, hemorrhage appeared more extensive and the perivascular component greater than in alveolar hemorrhage. Entrapped air bubbles were seen in alveolar hemorrhage produced by overloading the pulmonary circulation with blood. The occurrence of perivascular hemorrhage in the same section would suggest its origin *in situ*.

Discussion

Atelectasis and consolidation of the lung appear to favor development of perivenous hemorrhage. This is correlated with the frequent occurrence of perivenous and septal hemorrhages in stillborn infants and with the greater incidence of septal hemorrhage in newborn premature infants compared with newborn full-term infants, since atelec-

tasis lasts longer in premature infants.[4,5] Coryllos and Birnbaum[6] have shown that blood flow through an area of consolidation or atelectasis is less than that through an aerated lung; occurrence of large perivascular hemorrhages in areas of atelectasis and consolidation may be related to this observation.

It was not possible, by overloading the pulmonary circulation with blood, to produce massive alveolar hemorrhage without also producing perivenous hemorrhage in the dying rat. This may indicate that, when massive alveolar hemorrhage is seen alone, some factor other than pulmonary congestion alone is involved, such as direct capillary injury. Infection and kernicterus in infants were associated with massive alveolar hemorrhage without perivenous hemorrhage; this suggests that capillary injury may be present in these conditions.

Experimental Pulmonary Hemorrhage in Excised Lung of the Cat

Method. Cats were anesthetized with ether. The aorta was cannulated and saline solution was perfused through the inferior vena cava until the heart stopped beating. The trachea was tied, the thorax opened, and 10 cc. of a 10 per cent solution of sodium nitrite was injected into the pulmonary artery. The heart and lungs were then removed en bloc. The pulmonary artery and left atrium were cannulated. The cannulas were attached to manometers. Blood was injected into either the pulmonary artery or vein by means of a syringe attached by a Y tube to cannula and manometer. In some animals bronchi and/or pulmonary vessels to one or more lobes were ligated before injection.

Results. Gross changes in the lungs were the same after arterial or venous injection. When blood was injected into the pulmonary circulation via the pulmonary artery or vein, the lungs became pink; petechiae appeared after 50 to 60 cc. had been injected, and dark red, firm areas of consolidation (shown microscopically to be areas of alveolar hemorrhage) appeared after 125 to 150 cc. of blood had been injected. This sequence of events occurred regardless of direction of blood flow or speed of increase in pressure. The major factor in producing petechiae and massive hemorrhage was the amount of blood injected and not the injection pressure or the suddenness with which pressure was increased during injection. At a pressure of 54 mm. of Hg, a slow injection of blood yielded petechiae only after 60 cc. had been introduced. At a higher pressure of 70 mm., with rapid injection, the introduction of 50 cc. of blood did not produce petechiae. Additional amounts of blood resulted in hemorrhage.

When the arterial side was injected, venous pressure rose slowly. When the venous side was injected, arterial pressure rose rapidly. When the vessels supplying an upper and lower lobe were ligated, thus decreasing the pulmonary vascular bed, injection of a smaller volume of blood, at a much lower pressure, was required to produce petechiae in the unligated lobes than was required to produce them in the "complete" lung. With a portion of the lung collapsed (bronchus tied with lung deflated and then the remainder of the lung inflated), petechiae occurred in the collapsed portion at about the same time as in the expanded portion. Massive red consolidation was more evident in the collapsed lobe. The amount of blood required to produce petechiae and massive hemorrhage in a collapsed lobe was about the same as that required for an aerated lobe.

Effect of *ANTU*, Anticoagulants, Increased Blood Volume, and Vigorous Breathing on Development of Pulmonary Hemorrhage in the Rat (Table I)

Material and Methods. Forty rats killed by ether anesthesia or by intracerebral injection of alcohol served as controls. Petechiae were present in the lungs in 10 per cent and pneumonia was present in 5 per cent. Bronchiectasis was seen in several. One and five-tenths to 2.5 cc. of a 1 per cent aqueous suspension of alpha-naphthylthiourea (ANTU) was administered intraperitoneally. Hydrothorax and pulmonary edema were produced in 4 to 8 hours. Heparin was given intravenously in the tail vein, in doses of from 200 to 1,000 units. At necropsy, clotting times were more than 2 hours. Dicumarol was given intraperitoneally in doses of 10 to 25 mg. every day for at least 4 days. The rats died or were used for experiments from 4 to 10 days after administration of dicumarol was begun. The most frequent cause of death appeared to be peritoneal hemorrhage originating at the site of injection. The prothrombin time, when determined, was longer than 1 minute. The prothrombin time in control rats averaged 15 seconds. Dicumarol was given for at least 4 days before other agents. If heparin and ANTU were given to the same rat, they were given together. Rats were given transfusions of blood obtained from donor rats of the same species. Sodium citrate, citric acid, glucose solution (ACD*), was used as an anticoagulant. From 5 to 8 cc. of blood, plus enough saline solution to make 10 cc., was injected into the tail vein. Heparin and blood, if given to the same rat, were given at the

* Each 100 cc. of solution contains 2.45 gm. of dextrose, 1.37 gm. of sodium citrate, and 0.50 gm. of citric acid.

TABLE I
Experimental Pulmonary Hemorrhage in the Living Rat

	Number of rats	Results			
		No hemorrhage	Hemorrhage		
			Petechiae	Small	Large
Series 1					
A ANTU (causing pulmonary edema)	8	7	1	0	0
B Heparin	2	2	0	0	0
C Dicumarol	14	9	5	0	0
Total	24	18	6	0	0
Series 2					
A ANTU + heparin	3	3	0	0	0
B ANTU + dicumarol	3	2	1	0	0
Total	6	5	1	0	0
Series 3					
A Transfusion	3	2	1	0	0
B Transfusion + heparin	6	2	4	0	0
C Transfusion + ANTU	6	6	0	0	0
D Transfusion + heparin + ANTU	4	4	0	0	0
Total	19	14	5	0	0
Series 4					
A Breathing against pressure	12	5	6	0	1
B Breathing against pressure + dicumarol	8	3	2	2	1
Total	20	8	8	2	2
Series 5					
A Narrowing of trachea (plastic tube)	8	1	5	1	1
B Narrowing of trachea + heparin	7	1	1	3	2
C Narrowing of trachea + ANTU	3	1	2	0	0
D Narrowing of trachea + heparin + ANTU	3	2	0	1	0
E Narrowing of trachea + transfusion	2	0	0	0	2
F Narrowing of trachea + transfusion + heparin	5	1	0	2	2
G Narrowing of trachea + transfusion + heparin + ANTU	1	0	0	0	1
Total	29	6	8	7	8
Series 6					
A Anoxia	1	0	1	0	0
B Anoxia + heparin	2	2	0	0	0
C Anoxia + ANTU	2	2	0	0	0
D Anoxia + heparin + ANTU	1	1	0	0	0
E Anoxia + transfusion	2	0	2	0	0
F Anoxia + transfusion + heparin	4	0	2	2	0
Total	12	5	5	2	0
Grand total	110	56	33	11	10

same time. Transfusions and ANTU were given at the same time to several rats. Others were given transfusions after dyspnea had been produced by ANTU. As shown in series 3 (Table I), hemorrhage was not produced when these agents were combined.

Breathing against pressure was accomplished by the method described by Drinker.[7] The rats were anesthetized with ether and a tracheal cannula was inserted and attached to a system of tubes and valves, forcing the rats to breathe against 9 to 12 cc. of water pressure. Oxycel* was used successfully as a hemostatic agent at the site of tracheotomy, even in rats given large quantities of heparin.

Narrowing of the airway was accomplished by using a polyethylene tube as a tracheal cannula. The lumen of the tube was the size of the outer circumference of an 18-gauge needle. ANTU was given either simultaneously with tracheotomy or at varying intervals up to 8 hours after tracheotomy.

Anoxia was produced by placing the animal in a 5 l. jar with soda lime, and with a flow of nitrogen regulated to give an oxygen concentration of from 10 to 15 per cent as determined by the oximeter.

Results. Apparently ANTU alone or in combination with other agents did not influence the development of pulmonary hemorrhage. ANTU probably produced pulmonary edema by increasing capillary permeability.[2,8] On the basis of these experiments, no conclusions could be drawn in general concerning the relationship of pulmonary edema[9] to pulmonary hemorrhage, but it was shown that increased capillary permeability and pulmonary edema *per se* did not influence the development of pulmonary hemorrhage.

Heparin used alone did not produce petechiae. Experiments in series 3 (Table I) suggested that, when the circulation was overloaded with blood, petechiae were produced more easily in the presence of anticoagulants than without them. Because such petechiae appeared old at necropsy, they probably were a direct result of massive transfusions given 24 hours or more before the animals were killed. When heparin was given in combination with narrowing of the trachea, the number of positive results was not increased but the hemorrhages produced were relatively larger than those produced by narrowing of the trachea alone.

Petechiae were seen more often in the lungs of animals treated with dicumarol than in normal animals. Dicumarol may produce its effect by prolonging the prothrombin time or by producing degenerative changes in the vessel wall, as reported by McCarter, Bingham, and

* Parke, Davis & Co., Detroit, Mich.

Meyer.[10] The latter effect could not be shown in the lung sections. The effect of dicumarol appeared to be similar to that of heparin.

Transfusion alone did not produce death. In rats which had been given transfusions, the liver and lungs were congested. Animals forced to breathe against pressure died in from 2 minutes to 3 hours after insertion of the cannula. Petechiae were seen as frequently in animals which died early as in those which died later. The only example of large hemorrhage caused by pressure breathing alone was observed after a period of 40 minutes. Rats receiving dicumarol and made to breathe under pressure developed hemorrhages in 15 minutes, but large hemorrhages were not significantly more frequent. Large hemorrhages were found more commonly in the upper lobes while petechiae were scattered throughout. In 2 cases large thymic hemorrhages were seen without hemorrhage in the lungs. Microscopically, petechiae and large hemorrhages were alveolar. Most large hemorrhages were in alveoli adjacent to the bronchi. The alveoli in these areas were often collapsed (Fig. 3). In animals dying without hemorrhage, alveoli adjacent to the larger bronchi often were collapsed also.

Animals which had a plastic tube in the trachea and did not receive drugs or transfusions died in from 5 minutes to 24 hours; most of them lived more than 12 hours. After several hours of vigorous breathing produced by tracheal cannulation, pink foam appeared in the plastic cannula, continuing until the rat died. At necropsy, petechiae were seen in most rats, and larger hemorrhages in a few. When larger hemorrhages did occur, other factors usually were present. The animal with small hemorrhages died in 5 minutes, probably from suffocation. The animal with large hemorrhages died in 27 hours. During insertion of the plastic tube this animal aspirated a small amount of blood, which clogged the tube a few minutes later. This resulted in slow, labored respirations. The blood was removed from the tube by suction and vigorous artificial respiration was given by means of a syringe attached to the plastic cannula. A few seconds after removal of the syringe, bright red blood appeared in the cannula. The animal survived this episode and was killed 27 hours later by the injection of formalin into the brain. Grossly, all lobes showed great hemorrhage (Fig. 4). Microscopically, old and recent hemorrhages were seen. Another rat, killed after an almost identical sequence of events, had been given heparin after tracheal cannulation. The findings at necropsy were similar in these 2 cases. Rats receiving transfusion and tracheal cannulation died 13 to 26 hours after tracheotomy, except for one without hemorrhage which died in 2 hours and was found to have extensive

pneumonia. When transfusion was given at about the time the plastic tube was inserted, and the animals died or were killed 24 hours or more afterward, the lungs showed recent and old areas of hemorrhage larger than those from either method alone. When heparin was given, the hemorrhages were still larger. When large hemorrhages were produced without transfusion (series 5, A and B, Table I), additional factors, such as vigorous artificial respiration, probably were important in initiating bleeding. The following sequence of events was suggested by these observations: (1) congestion of the lung produced by transfusion; (2) petechiae occurring as a result of congestion and vigorous breathing; (3) continuation of hemorrhage, as vigorous breathing continues; (4) ever-increasing hemorrhage, due to heparin, once bleeding is initiated.

Congestion appeared to be an important factor in the initiation of hemorrhage. When heparin was given in combination with narrowing of the trachea, hemorrhages were larger than those produced by narrowing of the trachea alone. Grossly, the large hemorrhages were larger than those produced by breathing against pressure. Large hemorrhages were not observed unless the rat survived at least 13 hours with the plastic tube in place. No difference was observed in the size of hemorrhages in animals killed and in those allowed to die. Recent pneumonia and congestion were present in most animals with large hemorrhage. Ten were studied microscopically. Alveolar hemorrhage predominated but perivascular hemorrhage was present also; rupture of the venous wall was seen in one and rupture of the capillary wall in another.

Anoxia produced death in an average of 3 hours. The character and rate of respiration appeared similar to that observed in rats with cannulation of the trachea. As noted in Table I, anoxia alone produced petechiae. When, in addition, transfusion and heparin were given, hemorrhages were larger. Anoxia has long been implicated in the development of petechiae in the lungs and other viscera. The mechanism by which petechiae are produced in anoxia is not known. Review of the literature disclosed no experiments proving that petechiae in the lungs produced by anoxia were anything other than agonal phenomena. Those seen at necropsy and produced experimentally were thought to have developed terminally. Since the capillaries of the lung receive their oxygen directly from the air in the alveoli,[7] it is difficult to understand how anoxia of these capillaries would be important in the formation of hemorrhage occurring after transfusion. Injection of the pulmonary circulation of excised cat lung produced petechiae on the

surface of the lung in direct proportion to the amount of blood injected. Petechiae were not observed in the lungs of animals dying of ANTU poisoning. Our observations suggested that petechiae are not always due to anoxia and that anoxia does not always produce petechiae. They suggested also that severe congestion alone may produce petechiae and larger hemorrhages. Further experiments must be carried out in order to evaluate properly the rôle of direct anoxia and congestion in the pathogenesis of hemorrhage from small vessels in the lung.

The Rôle of Infection in the Production of Pulmonary Hemorrhage

Method. Twenty-nine kittens from 6 hours to 3 days old, varying from 80 to 120 gm. in birth weight, were used in these experiments. Five were sacrificed as controls, none of which showed pneumonia or hemorrhage. Ten were given *Staph. aureus, E. coli,* and gastric content (which had been aspirated from the kitten's stomach through a small plastic tube) alone or in combination. One was given saline solution. Thirteen were given Baker's cat virus* alone. All agents were administered intratracheally, using a small needle and syringe. The *Staph. aureus* and *E. coli* used were isolated from sick infants. The kittens were kept with the mother cat until they died or were killed. In kittens given bacteria, necropsy was performed 2 to 6 days after infection and in those with virus, 2 days to 6 weeks after infection. Cultures were made from the lungs of all kittens at necropsy and impression slides of the lungs were made in those given virus. These were stained with Macchiavello's stain.

Results. Table II summarizes the results of these experiments. Hemorrhage was found in 9 kittens, 4 in which bacteria were given and 5 in which Baker's cat virus was given.

E. coli had been given in the first 4, either alone or in combination with *Staph. aureus* and gastric juice. One of these received *E. coli,* gastric juice, and *Staph. aureus* at the age of 2½ days and died 1 day later. At necropsy large well circumscribed, bright red, firm areas were seen in all lobes of the lung (Fig. 5). Microscopically, in these areas many alveoli were filled with erythrocytes and some contained phagocytes. Some alveoli were collapsed without hemorrhage. Asphyxial membranes were seen in some ducts and alveoli. Granulocytes were rare. No necrosis or congestion was present. A litter mate of

* Baker's cat virus was obtained in yolk-sac suspension from Dr. M. D. Eaton, Harvard Medical School. One group of kittens received this material before serial transmission in egg embryos and another group received a yolk-sac suspension of the virus after two series of fresh egg-embryo transfers. Dr. L. K. Kingsland made the transfers.

this kitten, given the same doses of the same agents at the same time, died 1 day later with severe pneumonia and congestion but without hemorrhages. One kitten receiving *E. coli, Staph. aureus,* and gastric juice died in 1 hour, probably of suffocation from bronchial obstruction caused by the agents injected. Hemorrhage and aspirated material were present in the alveoli and bronchi in this kitten. In one given gastric juice and *E. coli,* areas of hemorrhagic necrosis were seen in the lungs 2 days after injection. One kitten given only *E. coli* showed

TABLE II

Pulmonary Hemorrhage Produced by Intratracheal Infection in the Kitten

Agent given	Number of kittens	Results		Culture from lungs at necropsy
		No hemorrhage	Hemorrhage	
Controls	5	5	0	No growth
Escherichia coli alone	1	0	1	*E. coli*
E coli + gastric contents	1	0	1	*E. coli*
E. coli + *Staphylococcus aureus* + gastric contents	5	3	2	*E. coli, Staph. aureus*
Staph. aureus alone	1	1	0	*Staph. aureus*
Staph. aureus + gastric content	1	1	0	No growth
Gastric contents alone	1	1	0	No growth
Saline solution	1	1	0	*E. coli,* few
Baker's cat virus	13	8	5	*E. coli* in 8

irregular areas of pneumonia, with hemorrhage about the bronchi and vessels but little alveolar hemorrhage.

Gastric juice and *Staph. aureus,* alone or in combination, produced pneumonia without hemorrhage. Every kitten except one (killed at 6 weeks) given Baker's cat virus showed pneumonia. Elementary bodies were seen in lung impressions or Giemsa stains in 8 of the 13 kittens. In 5, hemorrhage was present in the lungs. Elementary bodies were identified in 4 of these and *E. coli* was cultured from 3. Also, in 3 kittens without hemorrhage *E. coli* was cultured from the lungs at necropsy. Grossly, congestion and atelectasis closely simulated hemorrhage in kittens dying 1 day after infection, at the age of 2 days. The microscopic picture of the pneumonia produced by Baker's cat virus was similar to that described by Baker.[11] However, in some kittens which died in 1 or 2 days the disease was mild, showing congestion and a few alveolar phagocytes with precipitate. Elementary bodies were seen in 1 of these. In cases of viral or bacterial infection, no correlation could be established between hemorrhage and age of infec-

tion. Three kittens, given virus when 6 hours old, were examined at necropsy 3 days later and were found to have extensive pneumonia, negative lung cultures, and no hemorrhage.

Discussion

Gastric juice alone did not produce hemorrhage, but in one kitten, dying 1 hour after intratracheal administration of gastric juice, *E. coli,* and *Staph. aureus,* petechial pulmonary hemorrhages were present. The short time required to produce hemorrhage in this case implicated the gastric juice more than the bacteria. Gastric juice may act either by digesting the surfaces with which it is in contact or by producing atelectasis (due to obstruction by milk curds), with subsequent pneumonia. Winternitz, Smith, and McNamara[12,13] described hemorrhagic necrosis in the lungs of rabbits, caused by intratracheal injection of hydrochloric acid. Since necrosis was not seen in the hemorrhagic areas of lung in the kitten described, suffocation seems the best explanation for the hemorrhage. These experiments show that *E. coli* is capable of producing pneumonia with hemorrhage in kittens. However, in one case hemorrhage was produced with little cellular exudate and without necrosis in the lung.

Hemorrhage was not a prominent feature of the pneumonia produced by Baker's cat virus. Since the isolation of *E. coli* from the lungs of some of these kittens at post-mortem examination may have been important in producing pneumonia or hemorrhage, further investigation will be needed. Pneumonia was found incidentally in several rats, mentioned previously, in which a plastic tube had been inserted into the trachea and to which transfusions had been given. Areas of pneumonia were seen associated and unassociated with hemorrhage, and pneumonia appeared to be independent of the hemorrhage itself. Thus, when pneumonia and pulmonary hemorrhage are seen together, hemorrhage should not be attributed necessarily to pneumonia.

Summary

The lobular distribution of aspirated blood in the dependent portions of lungs helps to differentiate it from hemorrhage. Microscopically, entrapped air bubbles are seen more commonly in aspirated blood, but do occur frequently in hemorrhage produced by trauma and by overloading the pulmonary circulation with blood. The presence of perivascular and peribronchial bleeding associated with alveolar blood is circumstantial evidence indicative of alveolar hemorrhage. Alveolar hemorrhage, perivenous hemorrhage, and periarterial hemorrhage occur in the order named when the pulmonary circulation is overloaded

with blood. Perivascular hemorrhages are relatively more extensive in atelectatic than in aerated lungs. The site of hemorrhage is not dependent on the direction of blood flow. Petechiae and massive hemorrhage occur in the excised cat lung in direct proportion to the amount of blood injected into the pulmonary circulation. Pulmonary edema produced by alpha-naphthylthiourea (ANTU) does not influence the development of pulmonary hemorrhage in rats. Heparin and dicumarol, used in combination with other factors, produce larger hemorrhages than are produced without these agents. Dicumarol alone may produce small pulmonary hemorrhages but heparin alone does not. An increase of about 50 per cent in blood volume produces only petechiae in the lungs. Vigorous breathing, alone, produces petechiae in the lungs, but when it is combined with transfusion, hemorrhages are larger and more frequent. Petechial hemorrhages are not always due to anoxia and anoxia does not always produce petechiae. Severe congestion alone may produce petechiae and larger hemorrhages. In kittens, aspiration of *Escherichia coli* alone, or in combination with gastric juice and *Staphylococcus aureus*, may produce pulmonary hemorrhage and pneumonia, or hemorrhage alone. When pneumonia and hemorrhage are seen together in the lung, the hemorrhage should not necessarily be attributed to pneumonia.

REFERENCES

1. Ahvenainen, E. K., and Call, J. D. Pulmonary hemorrhage in infants: a descriptive study. *Am. J. Path.*, 1952, **28**, 1–23.
2. Latta, H. Pulmonary edema and pleural effusion produced by acute alpha-naphthyl thiourea poisoning in rats and dogs. *Bull. Johns Hopkins Hosp.*, 1947, **80**, 181–197.
3. Haynes, F. W., Kinney, T. D., Hellems, H. K., and Dexter, L. Circulatory changes in experimental pulmonary embolism. *Federation Proc.*, 1947, **6**, 125–126.
4. Farber, S., and Wilson, J. L. Atelectasis of the new-born; a study and critical review. *Am. J. Dis. Child.*, 1933, **46**, 572–589.
5. Ahvenainen, E. K. On changes in dilatation and signs of aspiration in fetal and neonatal lungs. *Acta paediat.*, 1948, **35**, suppl. 3, 125 pp.
6. Coryllos, P. N., and Birnbaum, G. L. The circulation in the compressed, atelectatic, and pneumonic lung (pneumothorax—apneumatosis—pneumonia). *Arch. Surg.*, 1929, **19**, 1346–1423.
7. Drinker, C. K. Pulmonary Edema and Inflammation: an Analysis of Processes Involved in the Formation and Removal of Pulmonary Transudates and Exudates. Harvard University Press, Cambridge, Mass., 1945, 106 pp.
8. Chanutin, A., Gjessing, E. C., and Ludewig, S. Alpha naphthylthiourea (ANTU) in dogs: electrophoretic and cholesterol studies on blood plasma and pleural effusion. *Proc. Soc. Exper. Biol. & Med.*, 1947, **64**, 174–179.

9. Henneman, P. H. Acute pulmonary edema, with special reference to experimental studies. *New England J. Med.*, 1946, **235**, 590–596; 619–624.

10. McCarter, J. C., Bingham, J. B., and Meyer, O. O. Studies on the hemorrhagic agent 3,3′-methylenebis (4-hydroxycoumarin). IV. The pathologic findings after the administration of dicumarol. *Am. J. Path.*, 1944, **20**, 651–659.

11. Baker, J. A. A virus causing pneumonia in cats and producing elementary bodies. *J. Exper. Med.*, 1944, **79**, 159–172.

12. Winternitz, M. C., Smith, G. H., and McNamara, F. P. Effect of intrabronchial insufflation of acid. *J. Exper. Med.*, 1920, **32**, 199–204.

13. Winternitz, M. C., Smith, G. H., and McNamara, F. P. Production of pulmonary infarcts by the insufflation of acid. *J. Exper. Med.*, 1920, **32**, 211–217.

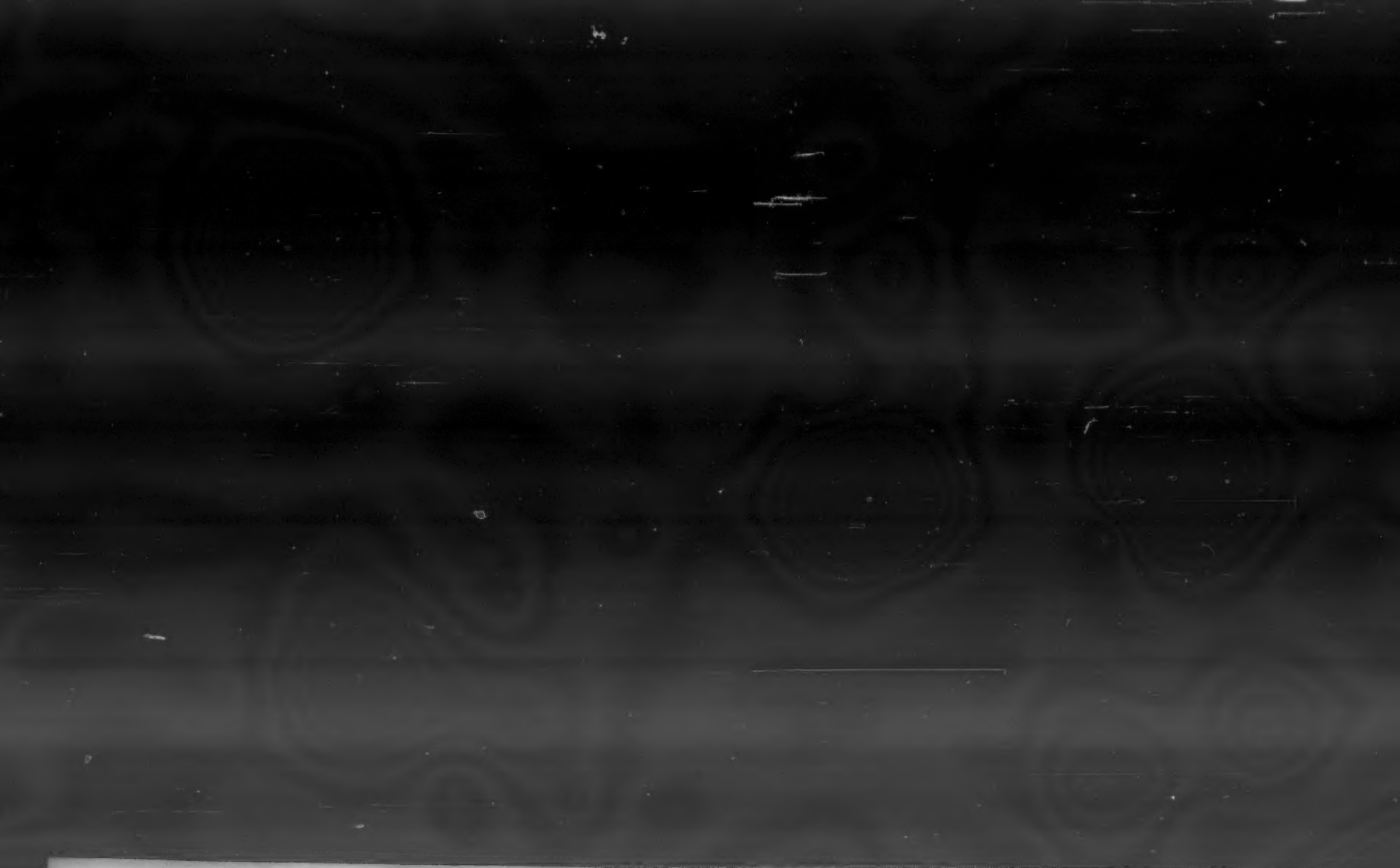
DESCRIPTION OF PLATES

PLATE 25

FIG. 1. Lobular distribution of aspirated blood. Rat was in the supine position when blood was injected.

FIG. 2. Extensive perivenous hemorrhage with minimal alveolar hemorrhage after injection of blood into the pulmonary artery of the collapsed rat lung. (The lungs were expanded after the injection.) Hematoxylin and eosin stain.

FIG. 3. Hemorrhage in collapsed alveoli of the peribronchial area in the rat after several hours of dyspnea produced by breathing against pressure. Hematoxylin and eosin stain.



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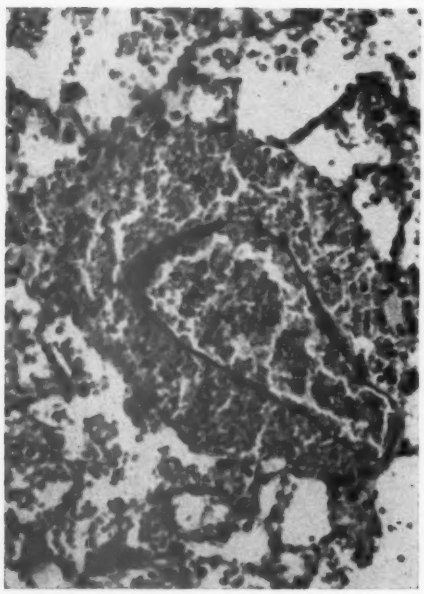

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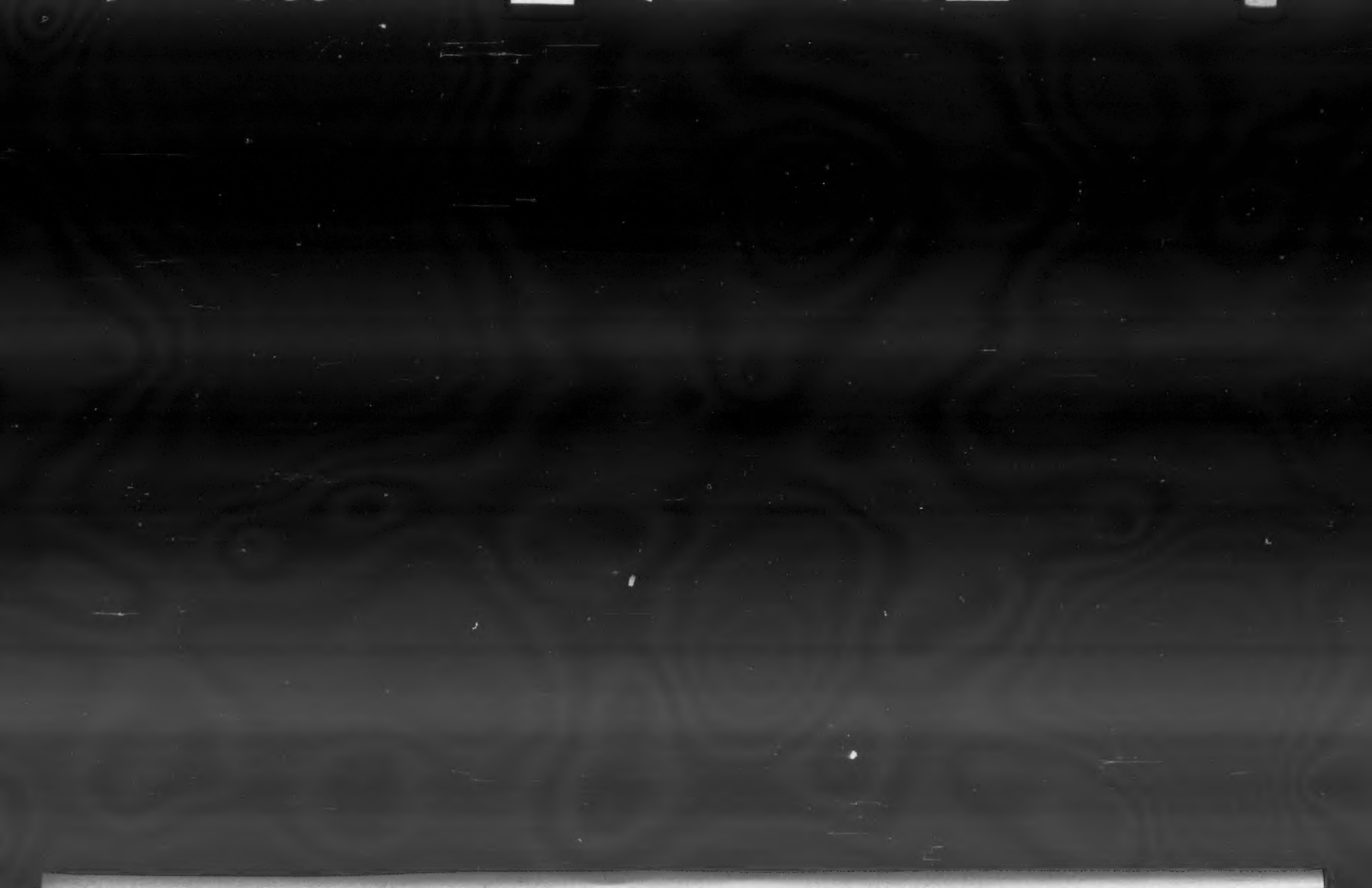
Ahvenainen and Call Pulmonary Hemorrhage, Experimental Studies

PLATE 26

FIG. 4. Massive hemorrhage produced in the lung of a rat 24 hours after narrowing of the trachea by insertion of a small plastic tube.

FIG. 5. Hemorrhage in kitten's lung 1 day after aspiration of gastric contents, *Escherichia coli*, and *Staphylococcus aureus*.



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Ahvenainen and Call Pulmonary Hemorrhage, Experimental Studies

EXPERIMENTAL ENDOGENOUS LIPOID PNEUMONIA*

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Discussions of lipoid pneumonia are generally concerned with chronic pulmonary inflammation due to lipids of exogenous origin. Robbins and Sniffen[1] recently described a significant deviation from this usual type in 11 instances of surgically excised pulmonary tissue in which a "chronic pneumonitis of cholesterol type" was found. Although bronchial obstruction was present in some of these cases, Mallory[2] indicated that such was not the case in all. It would seem that the associated lipids probably were endogenous even though the authors stated that the origin was unknown and that there was no evidence of formation locally.

Focal deposits of endogenous lipids are common in the walls of lung abscesses, or of bronchiectatic cavities. They also occur distal to bronchial obstruction and within macrophages in organizing pneumonia. A diffuse pneumonitis due to endogenous lipids has not been recognized heretofore, with the possible exception of the cases studied by Robbins and Sniffen[1] which were not associated with bronchial obstruction.

The purpose of this paper is to describe endogenous lipoid pneumonia which involved all lobes of both lungs and occurred with a very high incidence in experimental animals as an unexpected finding during an investigation into the toxicity of antimony trioxide.

Methods and Materials

An aqueous suspension of finely divided antimony trioxide was dispersed by a compressed air atomizer and the resulting suspension of droplets heat dried. The dust-laden air was then forced through an impinger to trap the aggregates and larger particles (greater than 1 μ) before being blown into an inhalation chamber housing 50 young male rats of the Sprague-Dawley strain. The chamber air was passed through an electrostatic precipitator before discharge to the outside. Electron microscope photographs of dust samples removed from the electrostatic precipitator indicated that the antimony trioxide dust had a mean particle size of 0.6 μ. The weight of the dust precipitated dur-

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ing a day's run divided by the total volume of air which passed through the chamber (determined by a flow meter) yielded 100 to 125 mg. of Sb_2O_3 per cubic meter as the average dust content of the air which the animals inhaled. The exposure averaged 25 hours per week and was continued for 14½ months.

As a second group, two control lots of 50 rats each were exposed simultaneously in identical inhalation chambers to dust in concentrations of approximately 25 mg. per cubic meter of air, and of the same particle size but containing only 1 per cent of antimony trioxide. The source of the compressed air and other experimental conditions were identical to those of the experimental group.

A third group of 30 rats was given intratracheal injections of aqueous suspensions of antimony trioxide (mean particle size, 1.5 μ) and then housed away from further contact with the dust. While the volume of each injection was 1 ml., the total weight of antimony trioxide injected varied from 75 to 125 mg. In a sense, this group constitutes another control in that it eliminated the inhalation apparatus.

Beginning at 2 months and at varying intervals thereafter, rats were removed from the inhalation chambers and sacrificed with ether, some immediately and others after holding periods which varied from 1 week to 12½ months. Some animals died of spontaneous pneumonia. The lungs of all rats were excised and expanded with formalin injected intratracheally under a head of 15 cm. Antimony determinations were made on formalin-fixed lung tissue by the method of Maren.[3]

Results

No significant difference in the gross appearances of the lungs from the various groups was noted until about the ninth month when the experimental group showed scattered, chalk-white, pleural foci about 2 mm. in diameter. With longer exposure this mottling increased in intensity and became associated with uniformly distributed fine depressions suggestive of orange skin. The cut surface showed similar mottling. When such a lung was stained *in toto* with Sudan IV, it took on a much deeper color than control tissue, and the white areas stained an even deeper red than the remainder. The cut surface was equally sudanophilic.

There were surprisingly few instances of grossly recognizable spontaneous pneumonia or bronchiectasis in the experimental group (9 deaths), whereas the control groups in the inhalation chambers showed a higher incidence of pneumonia and bronchiectasis with 14 and 18 deaths from each, respectively.

Microscopic Changes

The early microscopic changes in the lungs of the experimental group which inhaled the high concentration of antimony trioxide consisted of proliferation, swelling, and desquamation of alveolar lining cells. The dust was ingested rapidly by the alveolar macrophages. Similar cells were found in the interstitial tissue, forming dense pigment deposits in the peribronchial and perivascular stroma and also within some alveolar walls. These deposits were composed of densely aggregated pigment which was entirely intracellular. Such dense pigment also was seen in some of the macrophages lying in the alveolar spaces. Other nearby macrophages were filled with scattered, finely divided pigment granules. Pronounced proliferation and swelling of alveolar lining cells were accompanied by increasing tortuosity and branching of reticulum fibers in the thickened alveolar walls. These fibers tended to surround the lining cells as the disease progressed.

With longer exposure, fatty degeneration became increasingly evident in the alveolar macrophages. This was indicated by foamy cytoplasm and sudanophilia. The fatty degeneration, accompanied by severe swelling of the cells, progressed to necrosis and culminated in cell rupture. The cellular débris was ingested by a fresh crop of macrophages which likewise underwent fatty degeneration, necrosis, and rupture, adding more débris and lipoid material to the alveolar content. This débris, with finely dispersed antimony trioxide dust, might be loosely distributed or it might form dense acidophilic masses containing varying amounts of structureless, deeply basophilic material.

One of the most striking features of this disease was the occurrence in frozen sections of many colorless, needle-shaped crystals within alveoli which were choked with débris and swollen, fat-containing macrophages. These crystals were soluble in fat solvents and occurred in large and small sheaves as well as singly. An occasional small crystal might also occur within a macrophage. Many, if not all, of the crystals gave a positive Liebermann-Burchard reaction for steroid (cholesterol?). In frozen sections abundant fine and coarse sudanophilic droplets were found within the macrophages as well as in the intra-alveolar débris.

Varying degrees of collagenous thickening of alveolar walls and interstitial tissue were noted. In the early stages this was mild and occurred in isolated and widely scattered small foci. Later the foci, becoming more numerous and larger, tended to coalesce so that little uninvolved lung tissue was found in the terminal stage. There was

particularly dense collagenization about crystal spaces where the connective tissue was frequently of acellular, hyaline character.

The changes in the reticulum pattern kept pace with the collagenization. There were irregular and heavy condensations of reticulum in the regions of the most dense collagen deposits, particularly about crystal spaces. In general, the simple pattern of the normal expanded alveolus was lost and was replaced by arborescent masses of fibers which tended occasionally to obliterate alveolar spaces.

It is of interest that the amount of the interstitial deposits of antimony trioxide in the lungs was not proportional to the duration of exposure. After 14 months of exposure the lungs actually exhibited less interstitial, but more intra-alveolar, pigment than the lungs of rats exposed 9 months. This suggests that the interstitial deposits are not static.

Although the morphologic and chemical changes in the lung tissue were frequently severe, a distinctive feature was that inflammatory cells other than macrophages were absent with one minor exception. In several animals which died spontaneously toward the close of the experiment a few polymorphonuclear leukocytes were found scattered in the massive alveolar débris. This was in striking contrast to the spontaneous pulmonary inflammations of many of the control animals and some of the experimental animals in which lymphocytic, plasma cell, and polymorphonuclear exudation was prominent.

The satellite lymph nodes from cases of outspoken lipoid pneumonia contained antimony trioxide dust in dense deposits, most abundant in the cortex but also present in the medulla. The bulk of the pigment was intracellular but isolated, coarse, extracellular granules also were seen. No fibrosis or increased reticulum was demonstrable in relation to the pigment here. Since in most lymph nodes the tissue elements frequently were separated by edema, the absence of fixed tissue reaction was easily and decisively demonstrated.

Controls

The microscopic alterations in the lungs of the inhalation controls were minimal and consisted of mild proliferation of alveolar lining cells. A few scattered alveolar macrophages containing fine, black pigment granules and an occasional small cluster of macrophages with foamy cytoplasm completed the picture of the experimentally induced changes in these control animals. Lacking were the sudanophilic débris, lipoid crystals, collagenization, and alteration in reticulum pattern. Many of these lungs showed the spontaneous infections common

to ageing rats and characterized by purulent bronchitis, bronchiectasis, bronchopneumonia, and atelectasis.

The intratracheal controls which received the coarser particles of antimony trioxide in aqueous suspension showed microscopic lesions which were qualitatively identical to those seen in the experimental group. They occurred, however, in widely separated, relatively small foci. It is of interest that the typical fusiform crystal spaces, delimited by dense, heavily pigmented collagen, were found as early as 2 months following the intratracheal injection of the dust.

The antimony content of some of the lungs of the experimental group is given in Table I.

Discussion

In searching for an artefact or technical defect which might be responsible for the lipoid pneumonia, the inhalation of lubricating oil droplets from the air compressor was considered. The following facts eliminate the possibility of such an artefact and establish that the lipids are endogenous and intimately related to the inhaled or injected antimony trioxide:

1. No oily substance could be demonstrated, following prolonged exposure of white filter paper to the blast of air from the compressor.

2. The lipoid changes could not be demonstrated in the lungs of the inhalation control groups which were kept under the same exposure conditions as the experimental group except for a greatly reduced antimony content of the dust.

3. Lesions similar to those of the experimental group were produced by intratracheal injections of antimony trioxide.

4. The Liebermann-Burchard reaction establishes that some of the lipid is a steroid similar to, or identical with, cholesterol. (The recent work by Engle[4] suggests the need for caution in the interpretation of a positive Liebermann-Burchard reaction as definitely indicative of cholesterol.)

The absence of lipids and of fibrosis in lymph nodes containing deposits of antimony is intriguing. It is obvious that the alveolar macrophages, as living cells, can obtain nutrient material and can discharge waste products only by contact with the alveolar surface unless edema fluid is present. Failing such contact, the cellular metabolism suffers. One gains the first impression from a study of lung sections that many macrophages are suspended in air within the alveoli. This, of course, is not so since such cells could be kept in suspension only in edema fluid. Otherwise they will maintain contact with the alveolar wall due

to adhesiveness of the moist surfaces. If there are very many macrophages within an alveolus, however, some or many of them will have no continuous contact with the alveolar wall. Instead, many of the crowded macrophages will have contact with each other or, at best, only intermittent contact with the alveolar wall. On the other hand, if only relatively few macrophages are present within an alveolus, much of the free surface of the macrophages is brought in contact with the alveolar surface by the alternate collapse and expansion of the alveolar space. The phagocytic cells are thereby actively moved about and

TABLE I

*Antimony Content of Formalin-Fixed Lungs (Wet Weight)**

Rat no.	Exposure	Holding period	Weight of sample	Sb content	Average Sb content
	months	*months*	*gm.*	*γ/gm.*	*γ/gm.*
323	2	3	0.0708	1,306	1,306
329	2	4	0.1377	1,135	1,135
334	2	5	0.1978	367	367
343	2	5	0.0902	388	388
339	2	5½	0.1382	461	461
335	2	11	0.0902	292	292
345	2	12½	0.1305	393	393
363	9	0	0.1736	3,399	4,034
363	9	0	0.1659	4,665	
366	9	0	0.1911	3,807	
366	9	0	0.0902	6,430	
366	9	0	0.1014	1,430	2,795
366	9	0	0.1700	544	
366	9	0	0.2024	1,766	
369	9	0	0.0165	4,030	
369	9	0	0.0218	1,950	
369	9	0	0.0364	1,670	2,718
369	9	0	0.0282	3,220	
328	12½	0	0.3938	2,730	2,730
332	12½	0	0.2959	2,954	2,954
356	12½	0	0.1815	3,815	3,815
336	14	0	2.134	4,616	4,616
338	14	0	2.2746	7,237	7,237
346	14	0	0.0189	4,050	
346	14	0	0.0223	3,630	3,864
346	14	0	0.0209	2,970	
346	14	0	0.0180	4,805	
350	14	0	2.119	5,061	5,061
333	14	¼	3.229	3,107	3,107
354	14	¼	1.868	3,921	3,921
331	14	½	4.334	3,562	3,562
351	14	½	2.301	2,464	2,464
358	14	¾	1.6899	4,390	
358	14	¾	0.0122	5,150	
358	14	¾	0.0102	8,725	5,513
358	14	¾	0.0222	4,180	
358	14	¾	0.0170	5,120	
365	14	¾	1.5830	5,942	5,942

* The conversion factor for dry lung weight was found to be 4.85.

different portions of their surfaces are alternately brought into intimate contact with their source of nutrition and with inhaled particulate matter which is adherent to the alveolar surface. Thus, it is reasoned, the alveolar macrophage is more vulnerable to degenerative influences than is the macrophage within a lymph node which is continuously and entirely surrounded by its nutrient tissue fluid. A second point of difference, but of unknown significance, is the higher oxygen tension in the milieu of the alveolar macrophages as compared to that of macrophages within lymph nodes.

Regardless of whether this explanation is correct or not, the fact remains that the ingestion by alveolar macrophages of the relatively inert (chemically) and relatively insoluble antimony trioxide results in the accumulation of intracellular lipids and ultimate cell rupture. Much, if not most, of the fibrosis within the lung appears to be secondary to the lipids so liberated and acting as irritants. The absence of fibrosis in lymph nodes where heavy deposits of antimony trioxide are present may be considered evidence for the assumption that antimony trioxide does not directly cause pulmonary fibrosis.

This interpretation of the origin of the fibrosis is similar to the suggestion advanced by Fallon,[5] who explained the resemblance between the structure of a tubercle and that of a silicotic nodule on the basis that both are fibroblastic reactions evoked by phospholipids. In the first instance the lipid is derived from the capsule of the tubercle bacillus and in the second, from alterations in tissue metabolism induced by silica. Fallon demonstrated by chemical extraction that the phospholipid content of silicotic rabbit lungs increased in proportion to the severity of the experimentally induced silicosis.

Further consideration of these observations suggests certain interesting clinical implications. There is the possibility that lipidic substances may be associated with other forms of pneumoconiosis which are characterized by pulmonary fibrosis. (In lung sections from a case of pneumoconiosis due to bauxite fumes* we recently found much sudanophilic material associated with abundant needle-shaped crystals, soluble in fat solvents.) Another possibility is that some of the cases of clinical pneumonia not now fully explained[2] may be related to endogenous lipids.

Summary and Conclusions

The inhalation by rats of finely divided antimony trioxide particles (100 to 125 mg. per cubic meter of air) for periods of 2 to 14 months produced a lipoid pneumonia in a high percentage of these animals.

* We are indebted to Dr. G. R. Finlay for this tissue.

Antimony trioxide, finely divided (1 μ or less), in contact with the alveolar lining induced proliferation, swelling, and desquamation of alveolar lining cells. The antimony trioxide particles cause metabolic disturbances within macrophages which lead to their fatty degeneration and necrosis. Lipids derived from such cells are demonstrable as sudanophilic droplets and as crystals. The latter are soluble in fat solvents and give a positive reaction for steroid (cholesterol?). It is these lipids from the disintegrated macrophages which cause varying degrees of pulmonary fibrosis. The disease in rats is further characterized by a paucity or absence of inflammatory cells other than macrophages. The absence of fibrosis from lymph nodes which contain heavy deposits of antimony trioxide is considered evidence for the assumption that antimony trioxide does not directly cause pulmonary fibrosis.

Similar pulmonary lesions, though much less severe, were produced in rats by intratracheal injections of finely divided antimony trioxide (75 to 125 mg.) as aqueous suspensions.

Possible clinical implications are: (1) that other pneumoconioses may be associated with lipidic substances of endogenous origin; and (2) that certain clinical lipoid pneumonias not now fully explained may be related to endogenous lipids.

Acknowledgment is made of the technical assistance of Miss Nancy L. Butler and Messrs. Raymond P. Srsic and Raymond S. Clemens. Miss Marian Westrick made the antimony determinations on the lungs.

REFERENCES

1. Robbins, L. L., and Sniffen, R. C. Correlation between the roentgenologic and pathologic findings in chronic pneumonitis of the cholesterol type. *Radiology*, 1949, **53**, 187–202.

2. Mallory, T. B. Discussion of: Gross, P., Brown, J. H. U., and Hatch, T. F. Experimental endogenous lipoid pneumonia. (Abstract.) *Am. J. Path.*, 1951, **27**, 690–691.

3. Maren, T. H. Colorimetric microdetermination of antimony with rhodamine B. *Analytical Chem.*, 1947, **19**, 487–491.

4. Engle, R. L., Jr. Giant cell foreign body reaction to non-cholesterol lipid plate crystals. A case report with chemical studies. *Am. J. Path.*, 1951, **27**, 317–325.

5. Fallon, J. T. Specific tissue reaction to phospholipids: a suggested explanation for the similarity of the lesions of silicosis and pulmonary tuberculosis. *Canad. M. A. J.*, 1937, **36**, 223–228.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 27

FIG. 1. Subpleural region of fibrosis with obliteration of some alveoli. There is irregular fibrous thickening of alveolar walls associated with the presence of many crystal clefts. The alveolar spaces are filled with foam cells and their débris, so that few or no air-containing alveoli remain. There is a notable absence of inflammatory cells other than macrophages. × 95.

FIG. 2. Frozen section stained with Sudan IV. The alveolar spaces are filled with sudanophilic débris and colorless crystals. The crystals are disposed in sheaves or parallel masses, less often in single plates. × 130.

FIG. 3. Same field as in Figure 2, but with crossed polaroid films. × 130.

FIG. 4. Lymph node with deposits of antimony trioxide in cortex. There is no demonstrable tissue reaction to the pigment, which is entirely intracellular. × 130.

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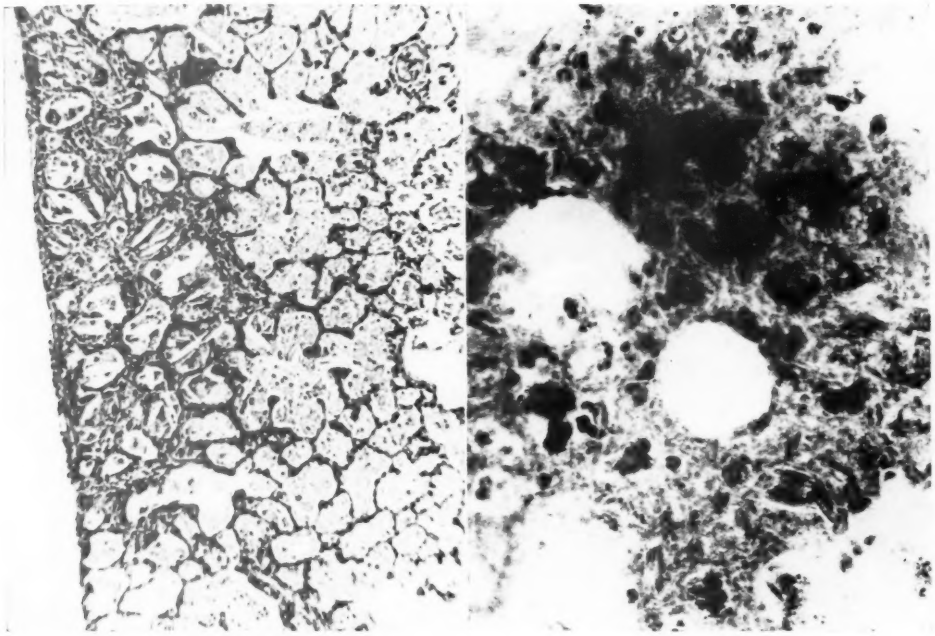

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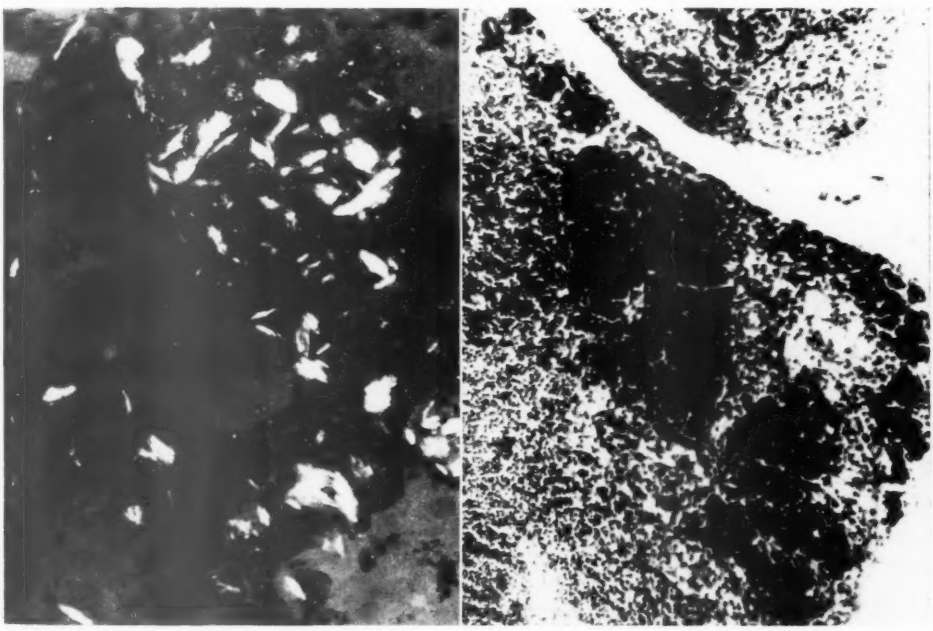

3 4

Gross, Brown, and Hatch Endogenous Lipoid Pneumonia

THE HISTOPATHOLOGY OF COXSACKIE VIRUS INFECTION IN MICE

I. Morphologic Observations with Four Different Viral Types*

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Coxsackie viruses appear to be among the most common infectious agents of man. Infection with these agents may mimic the symptoms produced in such diseases as non-paralytic poliomyelitis, aseptic meningitis, epidemic myalgia or pleurodynia (Bornholm disease), influenza, summer grippe, herpangina, or simply fever of unknown origin. These viruses are recognized by the ability to produce disease in infant mice, an infection which usually is manifested by paralysis. With some antigenic types, tremors and a marked ataxia are produced also. Although the histologic lesions produced by the virus in man are yet to be described, work on the lesions in the infant mouse has been carried out in several laboratories.[1-4]

In an earlier paper we have described the temporal relationship between the multiplication of the Connecticut-5 virus and the evolution of the muscle lesion.[1] It is the purpose of this present report to describe in detail the morphologic findings in mice infected with different Coxsackie viruses.

Methods

The viruses used for this report have been described elsewhere.[5-8] The source of each strain and the year in which it was obtained are indicated in Table I. Mice were inoculated as indicated, and usually sacrificed on the first day of perceptible disease. Animals generally were inoculated subcutaneously, although on occasion other routes were used.

For histologic study tissues were fixed in Zenker-acetic fluid (when decalcification was necessary) or in Zenker-formol or 10 per cent buffered formalin (pH 7). All tissues were sectioned at 6 μ after paraffin embedding. Sections were stained with hematoxylin eosin-azure, Heidenhain's iron hematoxylin, or Mallory's phosphotungstic acid hematoxylin stain. Masson's trichrome stain, Millar's stain for striped muscle,[9] and the Laidlaw silver stain were applied when necessary.

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Results

The over-all picture has been summarized in Table II, which lists the number of mice studied for each of the strains and the distribution of lesions in the tissues. The lesions in each tissue are discussed separately in the sections which follow.

MUSCLE

Necrosis of voluntary muscle is one of the most constant lesions of murine infection with any of the four types of Coxsackie virus used

TABLE I
Coxsackie Viruses Used in Present Experiments

Year	Antigenic type	Original source and reference	Area where strain originated
1948	Connecticut–5	Patient with aseptic meningitis[5,6]	New Haven, Connecticut
1947	Ohio–1	Patient with "summer grippe"[6,7]	Cincinnati, Ohio
1948	Texas–1	Flies[5,6]	Rio Grande Valley, Texas
1949	Easton–2 (Albany type 2)	Poliomyelitis patient[8]	Easton, Pennsylvania

in this study. Its extent and distribution vary with the strain of virus and the duration of the disease. The moderately myopathic Conn.-5 strain usually attacks first numerous scattered fiber segments only in selected muscles, which may be heavily involved as more fibers succumb with the longer duration of the infection. At the other extreme, the Texas-1 strain causes a devastating generalized or widespread myositis at the outset, in nearly all instances.

Degeneration. The striated muscle suffers an injury manifested by segmental necrosis of fibers, in the development of which several stages may be recognized. Among the earlier events is a widespread multiplication of both muscle and sarcolemmic nuclei, particularly in segments already showing the first evidences of injury. These are abruptly demarcated zones that appear slightly swollen and show darkened, broadened, and somewhat poorly defined A disks against a granular, intensely eosinophilic sarcoplasm, giving the effect of accentuation of the A disks (Figs. 1 and 7). The Z disks and sarcolemma remain unaltered. Sometimes, in Conn.-5 and Ohio-1 material, such fibers are found crumpled or folded in such a way as to suggest loss of function and passive plication by still active neighboring fibers. With the subsequent appearance of nuclear pyknosis in the affected segment, the A disks fade and become blurred, and the myofibrils darken, become

thicker, and fuse, forming undulant or straight longitudinal striae. The Z disks, which persist somewhat longer, also finally disappear at the end of this stage. The continuity of the altered myofibrils with the still delicate fibrils of the unaffected adjacent segments can be traced. These abnormal myofibrils usually are distinctly basophilic and fuchsinophilic, and lie in a matrix which may be eosinophilic or irregularly

TABLE II

Tissues Collected for Histologic Study and Distribution of Lesions

Age of animal at inoculation	Conn.–5		Ohio–1	Texas–1	Easton–2
	5 days	1 day	1 day	1 day	1 day
Muscle: No. of mice studied	61		20	43	14
Per cent with lesions*	47	70	70	96	80
Heart: No. of mice studied	35		16	39	28
Per cent with lesions	17	28	12	24	21
Fat: No. of mice studied	58		11	27	5
Per cent with lesions	83	60	64	0	0
Central nervous system: No. of mice studied	34		18	31	12
Per cent with lesions	61	17	28	0	0
Liver: No. of mice studied	27		11	10	20
Per cent with lesions	0	50	9	0	0
Pancreas: No. of mice studied	16		6	7	10
Per cent with lesions	0	61	0	?	0

* Per cent of specimens of each tissue showing lesions.

basophilic, so that the segment at this stage has a "combed" aspect. It sometimes occurs, particularly in Easton-2, Texas-1, and some Ohio-1 material, that these striae become selectively mineralized, in which case they stand out as coarse, beaded, parallel ribs stained a dark hematoxylin lake. As such, they may persist unchanged as an end stage after most of the surrounding matrix has disappeared. Usually, however, mineralization is slight or absent and the dense basophilic striae become thicker, more diffuse, and ultimately fade, leaving delicate trace lines. At that time karyorrhexis and karyolysis are advanced and the sarcoplasm expands, fuses, and shows local condensation. The degenerating segment then undergoes uniform hyalinization and forms a refractile, shortened, wax-like or vitreous mass seldom larger than 50 μ, with focal inhomogeneities, and is sharply delineated from the contiguous intact ends of the muscle fiber of which it formed a part (Figs. 2, 3, and 8). There usually is a continuous sarcolemma. These masses are clearly firmer, thicker, and more brittle than the adjacent intact fibers, which are locally compressed by the hyaline segments (Fig. 2). The nuclear remnants usually have disap-

peared. Comminution of the frangible hyaline segment may be accelerated by the action of still functioning muscles, creating stress fractures which tear the persisting sarcolemma and thus open a port for the entry of phagocytes. It is noteworthy that when almost universal muscular degeneration results, as with Texas-1 strain, and almost every muscle is involved, there is little tendency for the hyaline masses to fracture, and the sarcolemma remains largely intact, a circumstance believed due to the short explosive nature of the attack leaving little functionable muscle. This brings about a somewhat different unfolding of events than in the milder degrees of myopathy.

The tinctorial characteristics of the degenerating muscle change as regression proceeds. The homogeneous wax-like masses usually become less well defined, and show decreased affinity for stains. The mineralized segments remain strongly stained by hematoxylin. This process of degradation is rapid, and the number of hyalinized segments is at its peak on the first day of paralysis with all viral strains.

Regeneration begins soon after segmental necrosis, even before there is marked inflammatory reaction. Ingrowing sarcoblasts, connected by delicate strands of cytoplasm, insinuate themselves between the preserved sarcolemma and the hyalinized blocks (Fig. 3).

Inflammation. Inflammatory reaction follows closely upon the onset of fiber necrosis, and varies in quantity and distribution directly with the extent and location of muscle injury. Following the first localized appearance of segmental degeneration, hyperemia, edematous separation of fibers in the involved region, and small perivenular accumulations of mononuclear cells may be seen. With the extensive involvement of the muscle which is the rule on the first day of paralysis these changes become more marked, and there is an interstitial cellular exudate consisting chiefly of mononuclear (histiocytic) cells, some lymphocytes, and polymorphonuclear leukocytes. Hyperplasia of regional lymph nodes is the rule. Inflammation reaches its peak with the period of maximal tube formation (second day), and thereafter recedes slowly. Edema becomes extremely marked and the cellular exudate, now more abundant, is predominantly histiocytic. Two types are recognized: a large mononuclear cell with two or three karyosomes in a large, delicately outlined nucleus and abundant, vaguely defined, slightly basophilic cytoplasm; and a small mononuclear cell, similar to the monocyte. The massively affected muscles following inoculation of the Texas-1 strain are extremely edematous but have sparser cellular infiltrations. About these degenerated fibers the sarcolemmic sheaths are largely intact, and the ingrowth of regenerating slips fairly advanced.

Tube Formation. Débridement of the products of muscle degeneration is effected by the histiocytic phagocytes which enter the sarcolemmic sheaths enclosing the necrotic segments, and attack the hyaline masses. This is a process which generally follows very closely upon the onset of regenerative activity, and which proceeds concomitantly with the ingrowth of sarcoblastic elements. The resulting structures, in which a mixture of phagocytes, regenerating muscle cells, and remnants of hyaline material are all enclosed within the expanded sarcolemma or its remnants, has been described long ago (Sarcolemmschlauche of Waldeyer[10]), and may be designated "sarcolemmal tubes" (Figs. 3, 4, 9, and 10). Crumbling and fissuring of the altered hyaline blocks usually precede the entry of histiocytes, which seems to depend upon an opening in the sarcolemma, although the rent may not be seen in all instances. Histiocytes often are accumulated outside of an opening in the sheath, through which they may enter in file. The sarcolemma also may occasionally disappear over areas, exposing the naked block. The contracted hyaline masses contained within their preserved sarcolemmic sheaths, seen particularly in the Texas-1 strain lesions, are for a long time seemingly proof against the incursion of phagocytes, while the ingrowth of delicate sarcoblastic slips along the inner surface of the tube, prevented from collapsing by the presence of the waxy blocks, becomes striking.

The mononuclear phagocytes, having gained access to the interior, make their way chiefly into the fissures of the degenerated masses. A spherical, optically empty vacuole soon appears in the hyaline substance about each phagocyte ("vacuolar degeneration") (Figs. 3 and 10). Under the influence of this lytic activity, the hyaline material becomes paler, and presents a lacy appearance as the vacuoles enlarge and multiply at the expense of the hyaline remnant. Once histiocytic ingress occurs, the phagocytes gain early predominance over the regenerating elements by increase through immigration and mitotic division *in situ*. Phagocytic ingestion as well as lysis of hyaline débris takes place, for in the older tubes many enlarged, round, mononuclear cells are encountered, whose cytoplasm is laden with coarse fuchsinophilic or slightly eosinophilic granules and fragments, or else distended by many vacuoles. These cells wander out of the sarcolemmic tube and may be found in some numbers in the interstitium, a trend which depletes the previously very cellular tube. Evacuation of the products of necrosis is far advanced by the third day of clinical signs.

Regeneration. The regenerating muscle originates as outgrowth from the interrupted ends of intact parts of muscle fibers, and to a minor extent also within the degenerated segment from those altered

nuclei which were spared lethal injury. The latter explanation must be invoked to explain the presence of unequivocal single sarcoblasts deep within early and extensively degenerated segments, when, as in the "Texas" material, intact muscle is not present in the vicinity. It is supported by observations of intermediate changes in the form of nuclei in the midst of degraded parts, in process of assuming the typical form of young sarcoblastic nuclei. These would appear to correspond with the "Muskelkörperchen," which have been described.[11]

However, effectual major regeneration proceeds from intact parts of fibers, which are present in some numbers even in the tremendously destructive lesion of the Texas strain. In preparation for this, there occurs a marked proliferation of muscle nuclei in linear pattern along the periphery on one side, and also at the interrupted ends of the fiber (Figs. 3 and 13). These still have the form of muscle nuclei in that they are large, round, and vesicular, with delicately outlined nuclear membrane enclosing two dot-like nucleoli, and finely divided chromatin. Beginning at the end of the fiber abutting upon the destroyed segment, the nuclei rapidly alter, becoming elongate, fusiform, or ovoid with a heavily outlined, densely stained, nuclear membrane. Each has a few faint chromatin skeins and two or three very large, intensely basophilic nucleoli, one of which is characteristically rod or comma shaped, and the other thick and chunky. As the nuclei thus change, clear basophilic cytoplasm is gradually gathered immediately about them, and the sarcoblast is formed.

The outgrowth of regenerating elements occurs in two ways. In the "peripheral type" thin, finely pointed slips or fibers having about one-fourth the width of the parent fibers emerge at the periphery of the old fibers and advance chiefly along the inner sarcolemmic membranes into the destroyed field. They have homogeneous basophilic cytoplasm and characteristic nuclei arranged seriatim (Fig. 11). At first, apparently single or double sarcoblastic cells may emerge and advance far from a sarcoblastic strand or from the parent muscle fiber, suggesting regeneration by individual myoblasts. Often, however, such cells are in visible continuity with their fibers of origin by means of a thread-like cytoplasmic strand. It is probable that this is always the case,[12] and that the outgrowth from intact muscle is a continuous ribbon. This syncytial pattern becomes very evident as regeneration progresses. Individual sarcoblasts without connections are formed from those nuclei within necrotic segments which have been spared.

The rarer "terminal" or syncytial outgrowth produces a gradually tapered "bud" whose proximal end is of the same caliber as the parent muscle fiber of which it is a continuous part. The nuclei usually are

irregularly dispersed in the homogeneous basophilic cytoplasm, but may show a tendency to alignment. This form of regeneration occurs most often from the interrupted end of a fiber whose sarcolemma is continuous with the intact sheath of a degenerated segment, either empty or enclosing a solid hyaline mass, but it may also be simulated in other situations. That the growth of the sarcoblastic fibers is directed and guided by the persisting sarcolemmic sheath is obvious in the earlier phases as the pointed slips migrate along the inner surfaces of the open tube (Fig. 10). After phagocytic removal of the degenerated parts the sarcolemmic remnants tend to collapse or disappear and the guide lines for the continued prolongation of the now abundant and closely spaced new fibers are less readily apparent. In this florid growth regenerating slips usually are separated by the receding inflammatory exudate (Fig. 11), and in the expanded interstitial spaces thus made visible there is a considerable increase and alignment of fibroblasts parallel to the direction of growth, suggesting that the endomysial planes become important as directional lines.

From the early stages, bifurcation of regenerating slips arising from peripheral outgrowth can be recognized (Fig. 13). The method of the engagement of the branches into the parallel guide planes is not clear. They augment the rapidly increasing sarcoblastic mass whose growth rate in these young mice is extremely rapid. Regenerative activity, having just begun by the first day of overt disease, is well advanced by the second and third days and is, with the exception of final maturation, complete after 1 week. So exuberant is the propagation of these developing muscle elements, that on the third day of survival the previously defective area may resemble a myosarcoma (Fig. 5). It is remarkable that no mitotic figures unequivocally identifiable with the sarcoblasts can be made out in this intensively proliferating tissue. Many instances of mitotic division are found among the histiocytic cells in the interstitium and in the sarcolemmic tube; the origin of some mitotic figures cannot be established, but nowhere is karyokinesis to be seen in the rapidly growing and multiplying slips or their parent fibers. In the sarcoblastic slips the nuclei tend to lie in groups with squared-off, faceted, or congruous adjacent borders.

The average diseased muscle or muscle area which has survived to the third day presents great numbers of long (50 to 100 μ), slender, intensely basophilic slips with characteristic central nuclei in all stages of differentiation, a very cellular interstitial exudate and new fibrous tissues (Fig. 11). Scattered at frequent intervals there usually are segments of persistent adult muscle fibers each three or four times as thick as the offspring slip. It is evident that were each of the regen-

erating elements to gain maturity, the muscle would have many times its original bulk. That this does not occur must be due to the disappearance or the failure to mature of a considerable fraction of the sarcoblastic mass.

Differentiation. The earliest peripheral outgrowths are about 9 or 10 μ in diameter and their cytoplasm is clear and homogeneous, and stains a rich violet-blue with basic dyes. As early as the first day of clinical signs some of the longer (older) slips possess one or two definite unbroken longitudinal fibrils traversing their entire length. By the second day most of the sarcoblastic strands are equipped with two or three such fibrils in a parallel row on each side of the central nuclei. The nucleocytoplasmic ratio now decreases rapidly in the maturing fibers, and the more abundant sarcoplasm begins to accept a slight eosin tint. The longitudinal fibrils show regular periodic accentuations or thickenings, so that superficially they appear in strings of short dashes (Figs. 12 and 14). These represent the formative A disks. This development, seen in the more mature slips on the second day, is present in most of the new fibers on the third day; these fibers have now increased to about 15 μ in diameter, their nuclei are more widely separated, the nucleoli are less prominent, and the sarcoplasm stains mauve or purple with hematoxylin and eosin. The differentiating myofibrils lie peripherally, and the nuclei lie in a central column of clear, palely basophilic cytoplasm. In the further maturation of the fiber more myofibrils are continuously laid down; the sarcoplasm increases and becomes more eosinophilic as myoglobin is accumulated, and a definite sarcolemma appears; the nuclei revert to the form of mature muscle nuclei but remain central. The Z disk, seen on the fifth day, is the last development to be detected. At the end of 6 or 7 days, restitution of the devastated muscle is complete, save for certain minor residua, and only the slimness of the new muscle fibers and their central nuclei bear witness to their youth.

Regeneration leads to anatomical and apparently also to functional restitution of the muscle in a remarkably short time. Yet, long after paralysis there are persistent residua of the antecedent injury. In 2-day-old lesions caused principally by Texas, Easton, and sometimes Ohio-1 strains, mineralized masses composed of coarse parallel staves may be found in the interstitium, beset by some mononuclear cells and enclosed in very thin crescents or envelopes of cellular, fibrillar, new connective tissue. Occasionally non-mineralized inhomogeneous masses of partly basophilic hyaline material are found similarly enclosed in the interstitium in 3-day-old lesions due to Conn.-5 and Ohio-1 strains. In all cases there is slight or moderate, but definite, interstitial fibro-

blastic proliferation. The scattered interstitial masses would appear to be generally indigestible, and are dealt with as foreign bodies. In specimens surviving 11 days after Texas-1 virus infection, there are numerous scattered, intensely basophilic, ovoid masses beset by giant cells and surrounded by delicate connective tissue (Fig. 6). Most of the muscle fibers are slim, have central nuclei, abundant sarcoplasm, and are uniformly separated by a slight to moderate new growth of young fibrous tissue.

Discussion and Interpretation of the Myositis

The injury of the striated muscle caused by the Coxsackie viruses may be compared morphologically with the hyaline degeneration of Zenker. It is probable that the voluntary muscle fiber, like other highly specialized cells, responds only in a limited way to a great variety of differing noxa, reproducing a similar picture. Thus, hyaline or waxy degeneration of muscle has been associated with physical trauma, ischemia,[13] many febrile infectious diseases,[14-16] anaphylaxis,[17,18] chemical intoxication,[19] and particularly experimental nutritional deficiencies of Vitamin E[20-22] or potassium.[23] It would be speculative to attempt to assign a common fundamental denominator[24] to reconcile such divergent etiologic factors. Changes similar to those described have been found locally at the site of experimental intramuscular inoculation of certain neurotropic viruses (encephalomyocarditis Columbia-SK, equine encephalomyelitis, herpes) and mouse encephalomyelitis virus.[25] The viruses of the Coxsackie group, however, are consistently capable of giving rise, after intracerebral or intraperitoneal inoculation, to a widespread myopathy,[2] and rising titers of virus are recovered from the muscle.[1] But the encephalomyocarditis Columbia-SK virus which ordinarily yields only local lesions has been adapted by spleen passage to produce muscle lesions after intraperitoneal injection.[26] Such myopathic viruses seem to possess other well marked visceral affinities.[27]

Dalldorf[2,28] has distinguished, on the basis of morphologic differences, between the group A Coxsackie viruses which produce only generalized destruction of striated muscles, and the group B, in which the muscle lesions are focal and which is also neuropathic. The former would correspond with our Texas-1 and Easton-2 strains and the latter with our Conn.-5 and Ohio-1 strains. The differences in the muscle lesions produced by these types have been described in detail.

The selection of a certain muscle and of particular segments in a muscle fiber for destruction in Conn.-5 or Ohio-1 virus infection is governed by factors still wholly unknown. The Texas-1 and Easton-2

viruses, producing more widespread lesions, are definitely more myopathic. As in other organs, the recovery of rising concentrations of virus from muscle does not necessarily indicate that demonstrable lesions may be found in such muscle.[1] No inclusion bodies, such as were discovered with certain other myopathic viruses (mouse encephalomyelitis; encephalomyocarditis Columbia-SK)[25] were found.

Degradation of the fiber segment evidently proceeds through successive, rapidly ensuing stages to the final hyalinization. It is hardly possible to say whether the early nuclear multiplication, which precedes other visible injury, should be ascribed to an initial virus-induced stimulus to proliferation, or whether it represents the first evidences of regeneration, which begins so promptly. In viral myositis the two forms of muscle degeneration distinguished by Pfuhl[11] as "dissociative" and "true waxy change" would appear to be represented as injury merely of differing degrees of severity.

The rôle of the sarcolemma deserves particular mention. It is usually retained as a sheath, which is often clearly visible about the degenerated part quite distinct from the endomysium and becomes apparent when the sheath contents break up. In muscles with the scattered distribution of lesions characteristic of Conn.-5 or Ohio-1 viruses, discontinuities in the injured sarcolemma may be begun or accentuated by the tugging of adjacent functioning fibers. In Texas-1 material, perhaps because of the extent of the devastation, no such stresses are put upon the sarcolemmic sheaths, which remain largely intact, as protective scaffolds which permit a well marked degree of regeneration prior to inflammatory débridement of the field. Inflammation is apparently a consequence of necrosis, and the cellular component may be invoked by the products of degradation. The relatively acellular and fluid character of the early exudate in Texas-1 infection, in contrast to the prompt appearance of mononuclear cells in the Conn.-5 lesion, might be related to the protective ensheathment of the necrotic contents in the former instance, and their direct exposure in the latter. Tube formation is evidently delayed in the Texas-1 lesion, a fact which may also explain the large numbers of remnant masses in the surviving specimen.

The cellular constitution of the sarcolemmic tube and the rôles of the various cells drew the attention of Forbus,[29,30] who, unable to distinguish between phagocytes and muscle cells on morphologic grounds alone following Zenker's degeneration in human material, attempted to resolve the problem in experimental material with the aid of vital dyes.[30] Pfuhl's[11] careful studies of vitally stained preparations clearly differentiated between the dyed phagocytes and regenerating muscle,

which remains unstained. In these murine specimens, there is ordinarily no great difficulty in differentiating regenerating muscle elements from phagocytes, owing to distinctive nuclear configuration, basophilic cytoplasm, and mode of growth of the former. There is no evidence that the sarcoblasts are phagocytic, but the extent of their ingrowth in the absence of phagocytes suggests a sarcolytic function.

The development of phagocytic, histiocytic, or sarcolytic cells from sarcoblastic muscle elements, described in the older literature and by Forbus,[29,30] is said to have been observed directly *in vitro*.[31] Such transitions could not be traced in the recovering lesions of Coxsackie virus disease. Among the more interesting aspects of Coxsackie virus myopathy are the events of regeneration and differentiation. Infection with Coxsackie agents provides a useful and controllable method for the study of early regeneration and differentiation, since these processes proceed unimpeded in the face of viral concentrations which continue at high levels throughout the whole course of their evolution.[1] The occurrence of regeneration in striated muscles has been studied under a variety of circumstances,[10-12,14,29,30,32-35] and regeneration occurring after degenerations which do not interrupt the sarcolemma as in this case (designated embryonal), is known to differ in its anatomical details from that which occurs after profound traumatic interruptions of continuity. But these differences are more apparent than real, and are imposed by the preponderant growth of fibrous connective tissue in the latter contingency. Regeneration occurring along continuous sarcolemmic or endomysial sheaths, as occurs in the Coxsackie virus myopathy, leads to complete restoration. Modifications of the basic pattern may occur in different species[14,34] and in the same species at different ages;[32] but it is probable that in mammals the method of outgrowth of continuous sarcoblastic tips from the interrupted intact muscle represents the principal form of regeneration. The material of the present experiments affords no evidence that the regenerating muscle elements arise in any other way than by proliferation from the ends of uninjured muscle fibers, and, in this, most recent students agree. The concept supported by Levander[36] that new myoblastic elements may arise by differentiation of general connective tissue cells by a process of induction in the course of mammalian muscle regeneration, has yet to be confirmed.

The distinctive nuclear configuration of the early murine sarcoblasts is given by the large rod-like and massive nucleoli. Similar elongate nucleoli, called rhombosomes by Millar,[34] have been observed in the nuclei of regenerating rabbit muscle coincident with the first appearance of fibrils. The presence of the enlarged nucleoli of peculiar form

and the gathering of intensely basophilic cytoplasm are the morphologic reflections of intensive protein synthesis.[37] Indeed, a rate of growth and division so tremendous that it permits restitution by sarcoblasts of the whole bulk of a destroyed muscle in 3 days, and the early multiplication of differentiating myofibrils, must put great demands upon the protein synthetic mechanisms of the cell. Clark and Wajda[38] have estimated that regenerating rabbit muscle fibers grow at least 1 to 1.5 mm. per day, and it appears that in the infant mouse this growth capacity may be exceeded.

Failure to find mitotic figures in this rapidly proliferating sarcoblastic tissue, and the suggestive grouping of contiguous nuclei, oblige us to conclude that under the conditions of regeneration in these experiments the principal method of nuclear partition is amitotic. There is a difference of opinion on this point. Mitotic proliferation is said by some to occur in regenerating muscle of the rabbit[30] and mouse[25] while other recent observers of regeneration in man[29] and the rabbit[12,34] favor amitosis. Amitotic division in early stages and mitosis in the later stages are described as the methods of sarcoblastic reproduction by von Meyenburg.[14] Clark[12] brought important evidence to bear in an experiment in which he found no mitotic figures in the regenerating muscle of a colchicine treated rat, although there were many in the connective tissue elements.

The rôle of the sarcolemma and endomysium in directing and supporting the growth of regenerating slips, as described, has been stressed in the experiments of Clark[12] and the observations of Pfuhl.[11] Clark emphasized the importance of endomysial tube formation, which reinforces or reconstitutes the sarcolemmic sheaths, in the restoration of fiber architecture. If a piece of muscle be excised and replaced in a position at right angles to its original orientation, the regenerating fibers from surrounding muscle will make a right angled bend as they enter the graft, and come to be disposed transversely. According to Pfuhl, the sarcolemmic sheaths are very resistant, and when the young muscle fibers, with which they become filled, mature, the old sarcolemma disappears and new sarcolemma is formed; in his view, complete regeneration can occur only when the connective tissue of the muscle and the sarcolemma survive.

The cytodifferentiation of contractile elements in the sarcoblastic slips follows that described in normal embryonic myogenesis, with the appearance in order of homogeneous continuous myofibrils, A disk thickenings, and Z disk thickenings, which later join. The myofibrils are supposed to multiply by longitudinal splitting.[39] In Millar's

study[34] of rabbit muscle regeneration, the appearance of the Z disk preceded that of the A disk.

The exuberant growth of regenerating muscle proceeds not only in the presence of virus concentrations above infective levels,[1] but has been shown to progress independent of the nerve supply.[40] The degradation of the excessive sarcoblastic fibers is said to occur through atrophy or fatty change.[14]

The temporal aspects of the degenerative and regenerative phenomena following the injection of one strain of Coxsackie virus have been reported.[1] The sequence of events in these infant mice is more rapid than has hitherto been observed in other conditions, species, or in older mice.

The muscle lesion produced by the Coxsackie viruses is of dual interest in that it adds more data to the problems of neurotropic and viscerotropic virus variation, and the pathogenesis of lesions, and because it provides material for study of regeneration and differentiation.

HEART

Cardiac injury appears to affect only the myocardium, throughout which in the ventricles and sometimes in the atria there are scattered small patches of necrosis. Within these areas the fibers are homogeneous, condensed, hyalinized, and anuclear. They are separated by scant edema fluid and a very sparse infiltration of large mononuclear cells (Fig. 15). Calcification of necrotic fiber segments often takes place as early as the first day of signs of disease, and is found to be complete and uniform. In the few available myocardial lesions of animals surviving beyond the second day of signs, the necrotic muscle fibers appear vaguely defined, somewhat crumbled, and of reduced tinctorial affinity. They are now more widely separated, and there is interstitial fibrous proliferation, but the cellular exudate remains scant and of mononuclear type. No evidences of effective muscle regeneration could be recognized; neither were inclusion bodies found. The endocardium was intact in every case. Myocarditis was a feature of the lesions of all viral strains studied.

Discussion

Myocarditis has been produced experimentally with a number of viruses.[41,42] Focal myocardial necrosis is the rule in such experimental infections, in which a well marked cellular exudation usually has been noted also. A conspicuous inflammatory response and endocarditis were not features of the cardiac lesions caused by Coxsackie virus,

which, moreover, were different in nature and distribution from those described in the "spontaneous" cardiac lesions of mice.[43] The aregenerative character of the lesions after the second day of signs and 11 to 12 days after inoculation is in marked contrast to the active skeletal muscle regeneration taking place at the same time, and it would seem that effective myocardial regeneration cannot occur. This is known to be the case following experimental trauma and ischemia.[44]

CENTRAL NERVOUS SYSTEM

Central nervous system disease, produced either by intracerebral or intra-abdominal inoculation of the Coxsackie viruses of the Conn.-5 and Ohio-1 strains, is signalized during life by tremors and evidences of meningeal irritation. Anatomically, it is an acute encephalomyelitis affecting all parts of the brain, and attacking the cord with considerable frequency, but in which the brain stem is perhaps the chief site of action. Focal leptomeningitis occurs particularly in association with cerebral involvement. Lesions may be found diffusely scattered from cerebrum to cord, predominantly in the gray matter. There is marked generalized hyperemia, and perivascular accumulation of lymphocytic round cells which become more dense and abundant in the more advanced specimens (Fig. 17). Necrobiosis of dispersed groups of neurons, showing no specific or unusual features, is found in cerebral cortex, pons, mid-brain, hippocampus, and especially the medulla. Irregular early mineralization of such neurons is occasionally encountered. The conspicuous and often large areas of necrosis, occurring in both white and gray matter, are evident on the first day of sickness (9 days after inoculation and most marked with the intracerebral route) and result in complete devastation in areas. These zones of necrosis are generally demarcated and usually show little adjacent inflammatory reaction save for microglial activity, but in exceptional instances the local infiltration of polynuclear leukocytes and round cells may resemble a miliary abscess. The degenerated areas gradually rarefy, a process accompanied by mild reaction (Fig. 16). This progressive local encephalomalacia with loss of brain substance and atrophy encourages the development of hydrocephalus *ex vacuo*. Pappenheimer *et al.*[3] have traced the evolution of such lesions in surviving animals and their conversion into glia-lined cysts, resulting in "porencephaly," and there is no need to duplicate this description here.

Discussion

Central nervous system injury can be provoked by Conn.-5 and Ohio-1 viruses after intra-abdominal or intracerebral inoculation, the

latter route yielding a high incidence of lesions. The incidence and severity of encephalomyelitis, as indeed that of the muscle and fat lesions, increases sharply up to a point with increasing periods of viral residence in the host.[1] The observation made by Pappenheimer *et al.*[3] that animals with pancreatitis or hepatitis show no central nervous system lesions is also true of our material. This circumstance may be related to the fulminant nature and mortal effect of the former after shorter incubation periods than apparently are required for the development of encephalitis, which would seem to develop only after longer viral growth (6 to 9 days) and consequent higher titers, in the host.[1] It has been shown that after intra-abdominal inoculation the initial concentration of virus in the brain lags behind that of other tissues.[1] It may also be that the rarity of encephalitis in infection with the Texas-1 strain is due to the fact that after Texas-1 virus inoculations mice very rarely survive beyond the first day of signs, and there is therefore insufficient time for encephalitis to develop under these conditions.

Histologically, the central nervous system disease produced by Coxsackie virus bears closest analogy to the group of human arthropod-borne viral encephalitides (equine encephalomyelitis, Japanese B encephalitis).[45]

NECROTIZING STEATITIS

The unusual and characteristic lesions of necrotizing steatitis are found far more frequently in animals infected with Conn.-5 and Ohio-1 strains than are any other lesions, but are practically absent in mice inoculated with the Texas-1 or Easton-2 strains. A generalized destructive process occurs involving principally the maturing fetal fat lobules (interscapular pads, cervical and cephalic pads, at joints and rarely in the mesentery). To the unaided eye such fat appears swollen, grayish white, with opaque flecks and of mucoid consistency. Histologically, in the acute phase the usual details of fat lobules are completely smudged and in ordinary preparations each lobule presents a narrow outer band which stains with basic dyes, and an acidophilic or fuchsinophilic center composed of discrete embryonal fat cells. In the peripheral band the necrotic embryonal cells are vaguely outlined and usually fused; the reticulated cytoplasm is opaque, clouded, coarsely granular, stains with basic dyes, and encloses clear fat vacuoles of various sizes. A number of granules staining more sharply with hematoxylin, suggestive of mineralized material, are to be seen as early as the first day of clinical signs, and in exceptional instances massive mineralization has occurred on the second day (Fig. 20). The central or eccentric nuclei are pyknotic. In the inner zone of the lobule there

are many discrete cells with nets of coarsely granular eosinophilic cytoplasm containing small vacuoles, but also numerous groups of fused fat cells with uniform granular, eosinophilic, or fuchsinophilic cytoplasm and often distorted pyknotic nuclei (Fig. 18). Some fat cells are apparently always completely spared in this zone. The lobules are widely separated by marked edema, and in the interstitium there is an inflammatory cellular exudate consisting of polymorphonuclear and lymphocytic leukocytes and large mononuclear cells, the latter predominating.

In the further evolution of this lesion the inflammatory component plays the principal part. The outer basophilic fringe widens at the expense of the inner area, its cells are more diffuse and blurred, with a greater degree of mineralization, and the nuclei are either distorted and fragmented or have disappeared by lysis. Rounded bodies, varying from 25 to 100 μ, partly mineralized, amphophilic or basophilic, and with small vacuoles, appear in large numbers on the second or third day. They are vaguely outlined, and are derived from the anuclear cells and fused cell masses. At the same time infiltrating histiocytic phagocytes and lymphocytes become prominent within the lobules, particularly in the outer parts, and soon outnumber the recognizable altered fat cells and their remnants. There is conspicuous giant cell formation and active phagocytosis, most of the granular mineralized anuclear material being engulfed by phagocytes, which also contain small, clear, cytoplasmic vacuoles.

Moderate fibrous tissue proliferation, with the laying down of fine concentric lamellae in the lobular periphery, takes place after the third day. By the fourth to fifth day of signs definite large granulomata may be found within partially reconstituted lobules of mature fat (Fig. 19). Nevertheless, lobules at an early stage of necrosis are still present. The granulomata are usually stellate and occupy a large part of the lobule. They consist of closely set, large, histiocytic phagocytes and lymphocytes and giant cells, many containing the rounded basophilic bodies described. These are frequently wholly or partly mineralized, and a few are found lying free. There is definite fibrosis within and about the granulomas, around which there are the matured fat cells of the lobule.

Discussion

The generalized necrotizing steatitis of embryonal fat is a lesion unique to murine infection with certain of the Coxsackie viruses. This tissue is extremely susceptible to some viral strains (Conn.-5, Ohio-1, Powers, De Mole, McCarthy)[3] but relatively resistant to others (Texas-1, Easton-2). That the virus has a specific and injurious affin-

ity for adipose tissue is suggested, but not proved, by the recovery of virus from this tissue.[3] Panniculitis seen in animals is said to bear little resemblance to the lesions produced by the Coxsackie virus. The experimental and spontaneous steatitis of rabbits observed by Duran-Reynals[46] may be of viral etiology. It is interesting and perhaps of special significance that necrosis of striated muscle occurs in that disease.

In man, there are three disease entities affecting fat which bear histologic comparison with the advanced stages of the experimental lesion here described. While their obvious differences in behavior, incidence, and effects have compelled a nosologic differentiation among them, the essential lesions of localized lipogranuloma or traumatic fat necrosis,[47] relapsing febrile non-suppurative panniculitis (Weber-Christian disease),[48-50] and the rare adiponecrosis neonatorum,[51] are necrosis of fat cells and a granulomatous response.

An inflammatory process of the type described is apparently consequent upon direct injury to adipose tissue from various causes. It is the outstanding late lesion of the virus-induced affection of the fetal fat in mice, but the acute phases are notably different from the human lesions. The histogenesis of these lesions presents interesting and unsolved problems,[47,52] but while they would all appear to be morphogenetically analogous, their anatomical similarities obviously do not necessarily indicate a common cause. Among the numerous hypotheses concerning the causes of the obscure generalized affections of the fat tissue (Weber-Christian disease, adiponecrosis neonatorum), experience with the lipotropic property of certain Coxsackie viruses compels a closer examination of their possible viral origin.

PANCREAS AND LIVER

The very destructive nature of the lesions of the pancreas and liver appears to result in death very rapidly. Of the virus strains used in the present study, only the Conn.-5 was more or less consistently effective, the other strains producing such lesions only sporadically. Most remarkable is the limited age susceptibility, lesions being producible only if 1 or 2-day-old animals are inoculated.

The pancreatic lesion in its most aggravated form consists of parenchymal necrosis which spares the islets (Fig. 21). The pathology of this devastating pancreatitis has been discussed by Pappenheimer *et al.*,[3] and the alterations will not be described further here.

In general appearance, the hepatic lesion encountered in the present study was like that described by Pappenheimer *et al.*[3] in their experiments. There were all stages of degradation of liver cells, leading

ultimately to the formation of rounded, anuclear, homogeneous, hyaline refractile bodies with marked affinity for eosin (Fig. 22). These were often found lying free in the sinusoids or within Kupffer cells. The affected liver cell first shows nuclear hyperchromatism with changing of the chromatin. There ensues pyknosis and karyorrhexis, or karyolysis with displacement of the nuclear remnant, while the cytoplasm swells and becomes more eosinophilic and somewhat hyaline. Such cells, although still lying within a liver cord segment, are dissociated from neighboring cells. The anuclear hyaline bodies, many of which are found within segments of liver cords, appear to be formed after the loss of nuclear material. Their expulsion into the sinusoids might result from the total dissociation of the altered remnants of the liver cell cords. No hyaline bodies were found in any specimen in the complete absence of parenchymal necrosis.

Discussion

Besides the Conn.-5 strain, the Coxsackie viral strains known to produce pancreatitis and hepatitis regularly are those which have been designated Powers, De Mole, Kine, and Ohio-1.[3] A comparison of our experimental technic with that of the Harvard investigators and subsequent experiments which have been confirmed in both laboratories have established that only newborn, 1 and 2, or some 3 and 4-day-old mice are susceptible to viral pancreatitis or hepatitis, in the reverse order of frequency.

In an interesting group of experiments, Pappenheimer and his associates[55] have shown that the adult mouse also responds to the injection of a single infective dose of Conn.-5 virus with a destructive parenchymatous necrosis of the pancreas. In the adult animals, this is the only lesion producible, and in survivors, which have evidences of lack of exocrine secretion, there is found fatty replacement of most of the acinar tissue; the islets and smaller ducts are spared. From the observations of these investigators it would appear that only in the age period from about the 7th to the 21st days of life, comprising the period of nursing, is the pancreas relatively insusceptible to the necrotizing effect of the virus.

The viruses named share the capacity to produce necrotizing pancreatitis, as far as is known, only with the virus of mumps. Mumps pancreatitis, as seen in the very few fatalities, also may show massive parenchymatous coagulation necrosis.[53] That the islets may suffer in the course of mumps pancreatitis is shown by the subsequent development of diabetes in very rare cases. In infant mice with pancreatitis there is some evidence of lack only of exocrine secretions.

Viral hepatitis is better known, and while the changes herein reported are in some respects unique, they do resemble morphologically the hepatitis of yellow fever.[54] The rounded hyaline bodies described are seemingly analogous in appearance to the so-called Councilman bodies. Pappenheimer *et al.*[3] doubted that they originate from degenerated liver cells, and have demonstrated the presence of phospholipids like those of myelin, within them.

Comment

Some of the virus types comprising the Coxsackie group may be nearly as different in their pathogenetic capacities as they are antigenically. This has been recognized by Dalldorf[28] in his classification of the Coxsackie viruses into pathogenic types. In this scheme, group A is distinguished by causing generalized myopathy alone, and group B tends to produce both focal myopathy and central nervous system lesions. Our study of four immunologic types of Coxsackie virus (Conn.-5, Ohio-1, Texas-1, Easton-2) suggests that there is a compass of activity against susceptible tissues, ranging from the Conn.-5 strain, which provokes moderate muscle injury and severe lesions of the central nervous system, fat, liver, and pancreas, to the Texas-1 strain which produces the most severe muscle and cardiac damage, but rarely causes lesions of the nervous system, liver, pancreas, or fat. The Ohio-1 strain, while closer to the Conn.-5 type, would appear to be intermediate between these two. The pathogenetic tissue affinities of the various viral types, as manifested by the frequency and intensity of lesions, is summarized in Table III. A spectrum of tissue tropisms of various Coxsackie viruses has been charted by Pappenheimer *et al.*[3] Most of the viruses studied by the Harvard investigators showed marked visceral affinities, and most were reported as non-myopathic. In our experience, all of the Coxsackie viruses, comprising the four types named as well as over 60 untyped strains, produce myositis. It would appear that of all the tissues, the skeletal muscle is the one most consistently attacked by the Coxsackie viruses.

Except for focal pulmonary emphysema and atelectasis, lesions other than those described were not encountered.

Besides the strain of infecting virus, the age of the host is the most important factor modifying the nature and distribution of lesions. The rather narrow age limits of susceptibility of mice to the Coxsackie viruses are now well known[3,4] if poorly understood. Even more striking differences are to be observed in the apparent susceptibilities of different tissues at different ages within the period of infectivity. Hepatitis, which occurs in a high proportion (60 to 70 per cent) of

mice inoculated with the Conn.-5 strain during the neonatal period, cannot be induced by inoculation on or after the fourth day of life. After the fourth day of life suckling mice are refractory to viral pancreatitis and remain relatively insusceptible until adulthood.[55] Moreover, there is a progressively decreasing incidence of these lesions following inoculation on the second and third days, respectively.[5] Animals suffering from hepatitis or pancreatitis do not develop encephalitis[3] and involvement of other tissues is usually absent or milder,

TABLE III
Relative Pathogenicity of Four Strains of Coxsackie Virus for Various Tissues

Viral strain	Muscle		Heart		Central nervous system		Fat		Liver‡		Pancreas‡	
Conn.-5	61*	++†	35	++	34	+++	58	++++	27	++	16	++
Ohio-1	20	+++	16	+	18	+++	11	+++	11	+	6	−
Texas-1	43	++++	39	++	31	−	27	−	10	−	7	−
Easton-2	14	++++	28	++	12	−	5	−	20	−	10	−

* Number of specimens studied.
† Denotes incidence and severity of lesions, from least to greatest.
‡ Only newborn mice inoculated.

circumstances perhaps explainable by the shorter survival periods of such mice. It has been indicated[1] that with longer viral residence in the host up to a point, there is progressively higher incidence of lesions in muscle, nervous system, and fat. This rule seems also to be true of the severity and extent of lesions. Animals developing the more severe and widespread muscle, central nervous system, myocardial, and adipose tissue lesions are those which have survived longer and have thus proved capable of sustaining viral growth for a longer period before succumbing. This effect can be brought about by inoculating mice of an age (4 to 5 days) still within the limits of general susceptibility to the Conn.-5 type, but beyond that of hepatitis or pancreatitis. It is also of interest that adipose tissue retains its susceptibility to the necrotizing effect of certain Coxsackie viruses beyond the ages of susceptibility of other tissues.[3]

The ultimate explanation for the observed changes in age susceptibility of these particular tissues, differences which have been noted also with other viruses grown in the chick embryo, must relate the biochemical events of cellular differentiation to viral adaptation and pathogenicity, to the mutual illumination of both.

Although the histopathologic classification of Coxsackie virus, as suggested by Dalldorf,[28] into groups A and B may offer some advantages, it is much more laborious and uncertain than one based on anti-

genicity alone. Also there are gradations among the types which produce lesions outside of muscle tissue from Conn.-5 to Ohio-1 to Nancy. In fact the last is often difficult to distinguish from a typical myopathic strain like Texas-1. We have seen strains which at times produce in some mice only myositis and in others only limited cerebral lesions. To determine the distribution of lesions in mice, one must take into account such factors as age, route of inoculation, strain, and dose, and even then large numbers of mice have to be studied when each of these factors is varied. None of these variables can influence a classification which rests solely on an antigenic basis. The possibility should be kept open that we are dealing with different viruses, rather than different members of a family.

Summary

Coxsackie virus infection of infant mice characteristically results in necrotizing lesions of striated muscle and myocardium, and with certain strains, lesions in the central nervous system, liver, pancreas, and fat tissue. There are recognizable differences in pathogenicity of the four antigenic types studied (Connecticut-5, Ohio-1, Texas-1, Easton-2).

In the striated muscle there results segmental hyaline degeneration, in the morphogenesis of which successive antecedent stages are recognized. Inflammation occurs consequent to necrosis. Clearance of the products of injury is effected by histiocytic phagocytes through sarcolemmic tube formation. Regeneration, through the proliferation of sarcoblastic slips from intact muscle, begins very early and leads to restitution of the muscle within 1 week.

The heart lesion consists of focal myocardial necrosis.

Encephalomyelitis, often with focal meningitis, is found in Conn.-5 and Ohio-1 strain infections. It is characterized by zonal necrosis in both gray and white matter.

Lesions in liver and pancreas, producible by inoculation of newborn mice, are frankly necrotic.

In the maturing fat bodies there is found (in Conn.-5 and Ohio-1 material) a necrotizing steatitis in the evolution of which early mineralization and granulomata develop. This generalized affection of fat tissue by a known agent is believed unique. It can be compared with certain forms of panniculitis in man.

REFERENCES

1. Melnick, J. L., and Godman, G. C. Pathogenesis of Coxsackie virus infection. Multiplication of virus and evolution of the muscle lesion in mice. *J. Exper. Med.*, 1951, 93, 247–266.

2. Dalldorf, G. The Coxsackie viruses. *Bull. New York Acad. Med.*, 1950, **26**, 329–335.
3. Pappenheimer, A. M., Daniels, J. B., Cheever, F. S., and Weller, T. H. Lesions caused in suckling mice by certain viruses isolated from cases of so-called non-paralytic poliomyelitis and of pleurodynia. *J. Exper. Med.*, 1950, **92**, 169–190.
4. Melnick, J. L. Studies on the Coxsackie viruses: properties, immunological aspects and distribution in nature. *Bull. New York Acad. Med.*, 1950, **26**, 342–356.
5. Melnick, J. L., Shaw, E. W., and Curnen, E. C. A virus isolated from patients diagnosed as non-paralytic poliomyelitis or aseptic meningitis. *Proc. Soc. Exper. Biol. & Med.*, 1949, **71**, 344–349.
6. Melnick, J. L., and Ledinko, N. Immunological reactions of the Coxsackie viruses. I. The neutralization test: technic and application. *J. Exper. Med.*, 1950, **92**, 463–482.
7. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M. Ohio strains of a virus pathogenic for infant mice (Coxsackie group). Simultaneous occurrence with poliomyelitis virus in patients with "summer grippe." *J. Exper. Med.*, 1950, **91**, 185–195.
8. Melnick, J. L., and Kaplan, A. S. Dual antibody response to Coxsackie and poliomyelitis viruses in patients with paralytic poliomyelitis. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 812–815.
9. Millar, W. G. Observations on striated muscle. *J. Path. & Bact.*, 1933, **37**, 127–135.
10. Waldeyer, W. Ueber die Veränderungen der quergestreiften Muskeln bei der Entzündung und dem Typhusprozess, sowie über die Regeneration derselben nach Substanzdefecten. *Virchows Arch. f. path. Anat.*, 1865, **34**, 473–514.
11. Pfuhl, W. Die Regeneration der quergestreiften Muskelfasern nach wachsartiger Degeneration. *Ztschr. f. mikr.-anat. Forsch.*, 1937, **41**, 569–616.
12. Clark, W. E. L. An experimental study of the regeneration of mammalian striped muscle. *J. Anat.*, 1946, **80**, 24–36.
13. Schmincke, A. Die Kriegserkrankungen der quergestreiften Muskulatur. *Samml. klin. Vortr., Leipz.*, 1918 (n. F. No. 758–59). *Innere Med.*, No. 253–54, pp. 675–709.
14. von Meyenburg, H. Die quergestreifte Muskulatur. In: Henke, F., and Lubarsch, O. (eds.) Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1929, **9**, Pt. 1, 298–507.
15. MacCallum, W. G. The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917–1918. Monograph No. 10, Rockefeller Institute for Medical Research, New York, 1919, 147 pp.
16. Stenström, B. Degenerative changes in the skeletal muscles, particularly in infectious diseases. *Arch. Path.*, 1927, **3**, 361–373.
17. Beneke, R., and Steinschneider, E. Zur Kenntnis der anaphylaktischen Giftwirkungen. *Centralbl. f. allg. Path. u. path. Anat.*, 1912, **23**, 529–533.
18. Beneke, R. Weitere Beobachtungen über wachsige Muskeldegeneration nach anaphylaktischen Vergiftungen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1916–17, **63**, 633–648.
19. Walthard, B. Die pathologische Anatomie der Triorthokresylphosphatvergiftung. *Virchows Arch. f. path. Anat.*, 1948–49, **316**, 619–665.
20. Pappenheimer, A. M. The pathology of nutritional muscular dystrophy in young rats. *Am. J. Path.*, 1939, **15**, 179–183.
21. Pappenheimer, A. M. Muscular dystrophy in mice on vitamin E-deficient diet. *Am. J. Path.*, 1942, **18**, 169–181.

22. Tobin, C. E. Effects of vitamin E deficiency and cod liver oil on myopathy in mice. *Arch. Path.*, 1950, **50**, 385–392.
23. Smith, S. G., Black-Schaffer, B., and Lasater, T. E. Potassium deficiency syndrome in the rat and the dog. A description of the muscle changes in the potassium-depleted dog. *Arch. Path.*, 1950, **49**, 185–199.
24. Wells, H. G. The pathogenesis of waxy degeneration of striated muscles(Zenker's degeneration). *J. Exper. Med.*, 1909, **11**, 1–9.
25. Rustigian, R., and Pappenheimer, A. M. Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain other neurotropic viruses. *J. Exper. Med.*, 1949, **89**, 69–92.
26. Jungeblut, C. W., and Steenberg, E. Neurotropic and viscerotropic strains of Columbia SK and encephalomyocarditis virus. *Arch. Path.*, 1950, **49**, 574–581.
27. Jungeblut, C. W. Newer knowledge on the pathogenesis of poliomyelitis. *J. Pediat.*, 1950, **37**, 109–128.
28. Dalldorf, G. The Coxsackie viruses. *Federation Proc.*, 1950, **9**, 569.
29. Forbus, W. D. Pathologic changes in voluntary muscle. I. Degeneration and regeneration of the rectus abdominis in pneumonia. *Arch. Path.*, 1926, **2**, 318–339.
30. Forbus, W. D. Pathologic changes in voluntary muscle. II. Experimental study of degeneration and regeneration of striated muscle with vital stains. *Arch. Path.*, 1926, **2**, 486–499.
31. Chèvremont, M. Le muscle squelettique cultivé in vitro. Transformation d'éléments musculaires en macrophages. *Arch. de biol., Paris*, 1940, **51**, 313–333.
32. Barfurth, D. Zur Regeneration der Gewebe. *Arch. f. mikr. Anat.*, 1891, **37**, 406–491.
33. Volkmann, R. Ueber die Regeneration des quergestreiften Muskelgewebes beim Menschen und Säugethier. *Beitr. z. path. Anat. u. z. allg. Path.*, 1893, **12**, 233–332.
34. Millar, W. G. Regeneration of skeletal muscle in young rabbits. *J. Path. & Bact.*, 1934, **38**, 145–151.
35. Speidel, C. C. Studies of living muscles. I. Growth, injury, and repair of striated muscle, as revealed by prolonged observations of individual fibers in living frog tadpoles. *Am. J. Anat.*, 1937–38, **62**, 179–235.
36. Levander, G. Tissue induction. *Nature, London*, 1945, **155**, 148–149.
37. Caspersson, T. O. Cell Growth and Cell Function. A Cytochemical Study. W. W. Norton & Co., Inc., New York, 1950, pp. 101–140.
38. Clark, W. E. L., and Wajda, H. S. The growth and maturation of regenerating striated muscle fibres. *J. Anat.*, 1947, **81**, 56–63.
39. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1941, ed. 3, pp. 157–160.
40. Kirby, E. Experimentelle Untersuchungen über die Regeneration des quergestreiften Muskelgewebes. *Beitr. z. path. Anat. u. z. allg. Path.*, 1892, **11**, 302–319.
41. Helwig, F. C., and Schmidt, E. C. H. A filter-passing agent producing interstitial myocarditis in anthropoid apes and small animals. *Science*, 1945, **102**, 31–33.
42. Pearce, J. M. Cardiac Lesions Produced by Viruses. In: Kidd, J. G. (ed.) The Pathogenesis and Pathology of Viral Diseases. Columbia University Press, New York, 1950, pp. 107–133.
43. Gray, F. G. Spontaneous cardiac lesions in mice. Their bearing on attempts to produce experimental carditis. *Am. J. Path.*, 1949, **25**, 1215–1225.
44. Ring, P. A. Myocardial regeneration in experimental ischaemic lesions of the heart. *J. Path. & Bact.*, 1950, **62**, 21–27.

45. Wolf, A. The Pathology of Some Viral Encephalitides. In: Kidd, J. G. (ed.) The Pathogenesis and Pathology of Viral Diseases. Columbia University Press, New York, 1950, pp. 194–213.
46. Duran-Reynals, F. A necrotizing disease in rabbits affecting fatty and muscular tissues: analogies with Weber-Christian disease of humans. *Yale J. Biol. & Med.*, 1945–46, 18, 583–594.
47. Smetana, H. F., and Bernhard, W. Sclerosing lipogranuloma. *Arch. Path.*, 1950, 50, 296–325.
48. Christian, H. A. Relapsing Febrile Nodular Nonsuppurative Panniculitis (Weber-Christian Disease). In: Oxford Medicine, Oxford University Press, New York, 1949, 4, Pt. 4, pp. 804(55)–804(59).
49. Friedman, N. B. Fatal panniculitis. *Arch. Path.*, 1945, 39, 42–46.
50. Ungar, H. Relapsing febrile nodular inflammation of adipose tissue (Weber-Christian syndrome): report of a case with autopsy. *J. Path. & Bact.*, 1946, 58, 175–185.
51. Flory, C. M. Fat necrosis of the newborn. Report of a case with necrosis of the subcutaneous and visceral fat. *Arch. Path.*, 1948, 45, 278–288.
52. Lecène, P., and Moulonguet, P. La cytostéatonécrose ou saponification intracellulaire du tissu cellulo-adipeux sous-cutané. *Ann. d'anat. path.*, 1925, 2, 193–214.
53. Sabrazès, J., Broustet, P., and Beaudiment, R. Néphrite aiguë et pancréatite nécrosante suivie de mort, au cours des oreillons. *Gaz. hebd. d. sc. méd. de Bordeaux*, 1927, 48, 705–707.
54. Ash, J. E. Pathology of Yellow Fever. In: Kidd, J. G. (ed.) The Pathogenesis and Pathology of Viral Diseases. Columbia University Press, New York, 1950, pp. 134–151.
55. Pappenheimer, A. M., Kunz, L. J., and Richardson, S. Passage of Coxsackie virus (Connecticut-5 strain) in adult mice with production of pancreatic disease. *J. Exper. Med.*, 1951, 94, 45–64.

DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Striated muscle. Segmental necrosis in two fibers, illustrating early changes. The upper shows the earlier lesion: nuclear proliferation and pyknosis, accentuation of cross striations, and fissuring. The adjacent segment of muscle to the right has proliferated to form a regenerating strand with nuclei of typical form in linear series ("terminal regeneration"). The lower fiber shows a more advanced degree of degradation, with conspicuous crumbling of the hyaline blocks within the sarcolemma. Texas strain, first day of signs. Hematoxylin and eosin stain. × 600.

FIG. 2. Striated muscle. Hyaline or waxy change. The altered segments are firm and expanded, and compress the adjacent normal muscle (arrow). Connecticut-5, first day of signs. Hematoxylin eosinate-azure stain. × 500.

FIG. 3. Striated muscle. Segmental necrosis, inflammation, and sarcolemmic tube formation. In the center are sarcolemmic tubes in intermediate stages of development, continuous with the degenerated segments of which they form a part. Other hyaline segments are present in the same field, separated by elements of inflammatory exudate. Fusiform regenerating muscle slips (R) and mononuclear phagocytes (H) can be recognized and distinguished. Conn.-5, second day of signs. Hematoxylin-eosinate azure stain. × 500.

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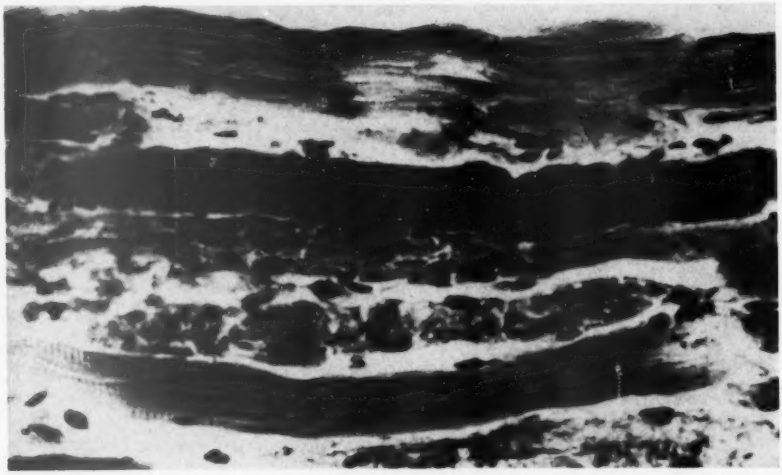

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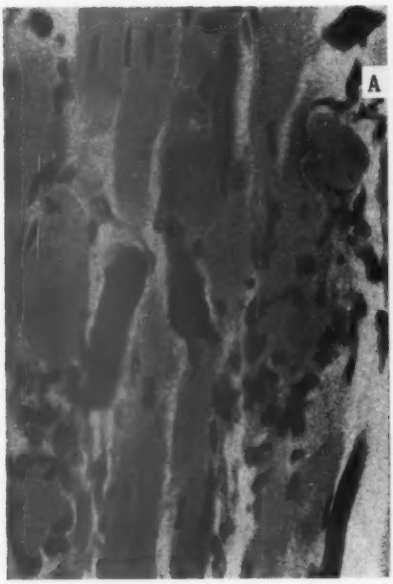

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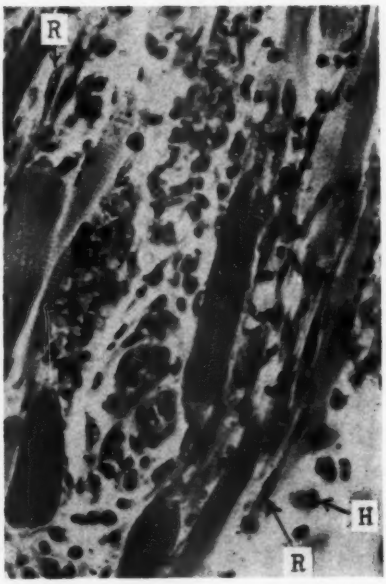

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PLATE 29

FIG. 4. Striated muscle. Two sarcolemmic tubes with numerous mononuclear phagocytes and remnants of hyaline material within the sarcolemmic sheaths. Sarcoblastic slips, originating from the intact muscle periphery, are growing into the lower tube. The demarcated segmental involvement is apparent. Conn.-5, second day of signs. Hematoxylin-eosinate azure stain. × 500.

FIG. 5. Striated muscle. Pseudo-sarcomatous appearance of muscle in regeneration after acute myositis. Ohio-1, third day of signs. Phloxine and methylene blue stain. × 100.

FIG. 6. Striated muscle. Partly mineralized residual masses surrounded by fibrous capsules, and sometimes by giant cells. The remainder of the muscle is completely restored. Texas, 11 days after onset of paralysis. Phloxine and methylene blue stain. × 200.

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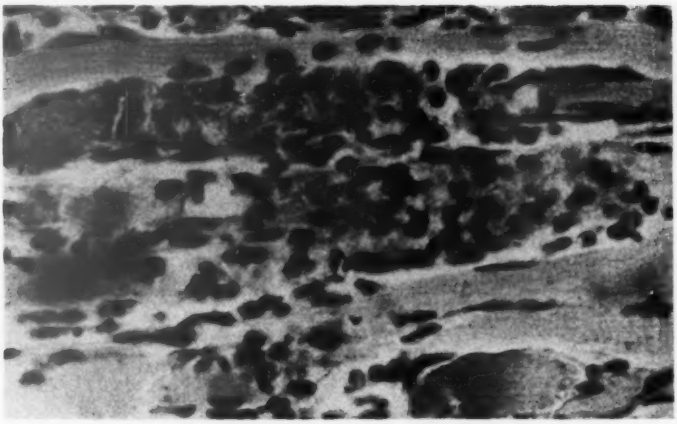

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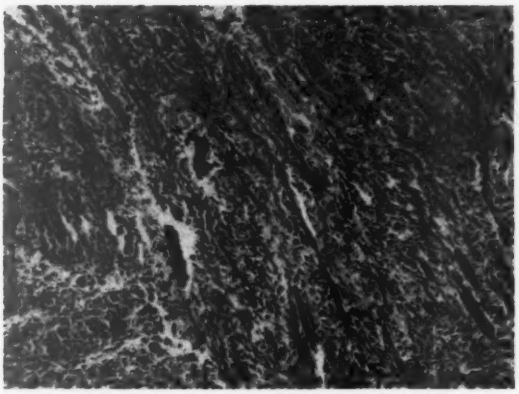

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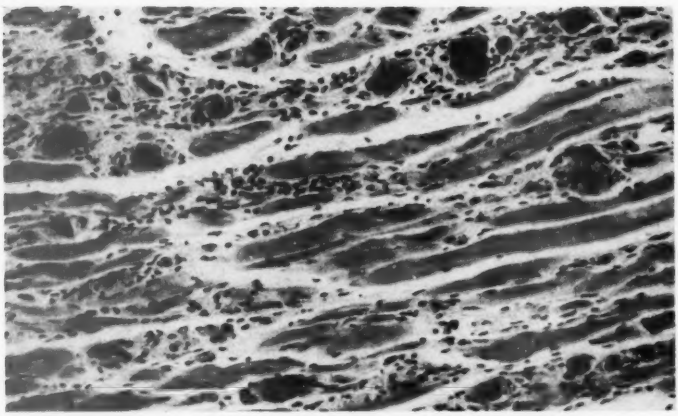

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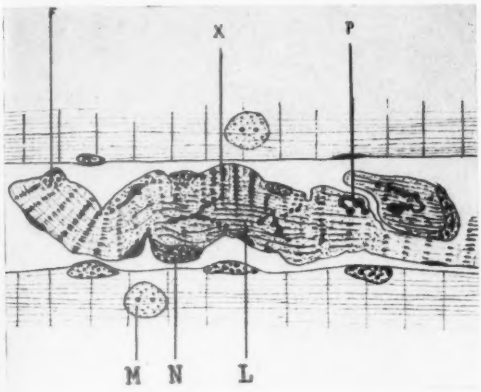
PLATE 30

FIG. 7. Striated muscle. Earliest appearance of segmental degeneration, showing irregular accentuation of cross striations (X), nuclear proliferation and pyknosis (P), or lysis (F). M = muscle nucleus of intact fiber, N = sarcolemmic nuclei, L = strial accentuation of myofibrils. Conn.-5, first day of signs. Hematoxylin and eosin stain. Freehand drawing, × 900.

FIG. 8. Striated muscle. Demarcated segmental degeneration in stage of hyalinization. Increased numbers of pyknotic nuclei (P) are present in the segment, which shows a fissure (F). H = hyaline material, N = muscle nuclei in intact part of fiber. Conn.-5, first day of signs. Hematoxylin and eosin stain. Freehand drawing, × 900.

FIG. 9. Striated muscle. Earliest stage of sarcolemmic tube formation, designed to illustrate the ingress of phagocytes and sarcoblastic slips into the demarcated segment of degenerated muscle. There are fissures in the hyaline material (H). P = distorted pyknotic muscle nuclei, S = sarcolemma, M = histiocyte, L = polymorphonuclear leukocytes, R = regenerating muscle elements, A = phagocyte with cytoplasmic inclusions. Ohio-1, second day of signs. Millar[9] stain. Drawn to scale, × 900.

FIG. 10. Striated muscle. Sarcolemmic tube at its highest development, showing vacuolar sarcolysis of hyaline material (H) by phagocytes (P) and ingrowing regenerating elements (R). S = sarcolemma, K = mitotic figure in histiocyte. For comparison with Figure 4. Conn.-5, second day of signs. Hematoxylin eosinate-azure stain. Freehand drawing, × 900.



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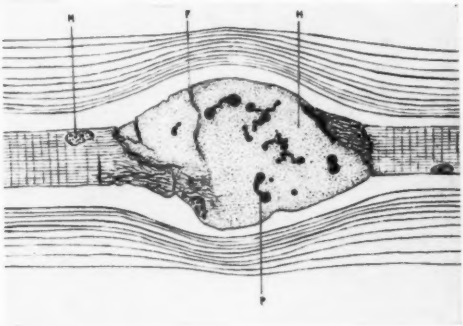

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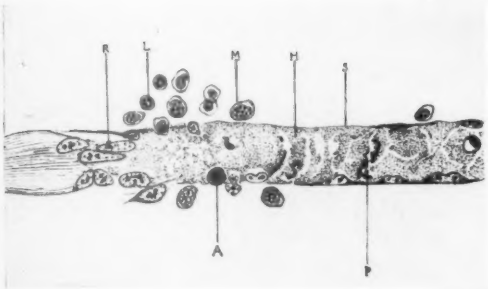

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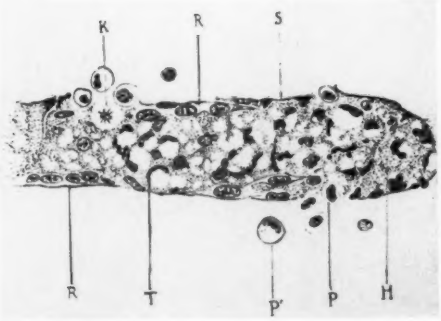

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PLATE 31

FIG. 11. Slips of regenerating and differentiating muscle, illustrating the slender plasmodial fibers and the typical nuclear alignment and configuration. The slips are separated by marked edema and an inflammatory exudate of mononuclear cells. Easton-2, second day of signs. Hematoxylin and eosin stain. × 400.

FIG. 12. A differentiating sarcoblastic slip, showing central nuclei and newly formed myofibrils in which periodic thickenings can be discerned. Iron-hematoxylin stain. × 900.

FIG. 13. Drawing to illustrate the "peripheral" type of regeneration (A) and the rare "terminal" type (B). M = muscle nucleus, resting phase. R = form of nuclei in regeneration. Hematoxylin eosinate-azure stain. Freehand drawing, × 900.

FIG. 14. Differentiation in the sarcoblastic slips, showing the appearance of myofibrils and the development of A disks, and the form of the central nuclei. Successive stages of differentiation are represented from above downward. Iron-hematoxylin stain. Freehand drawing, × 900.

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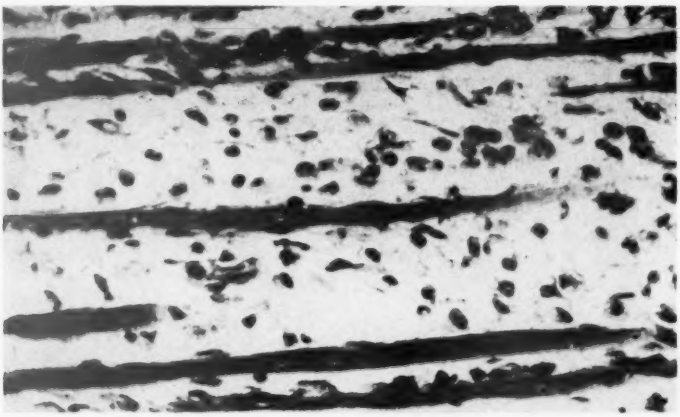

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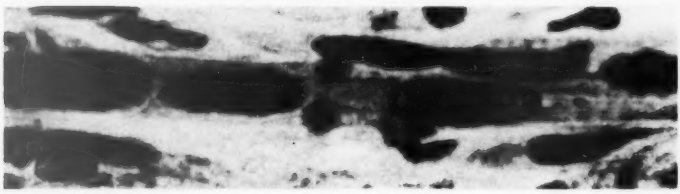

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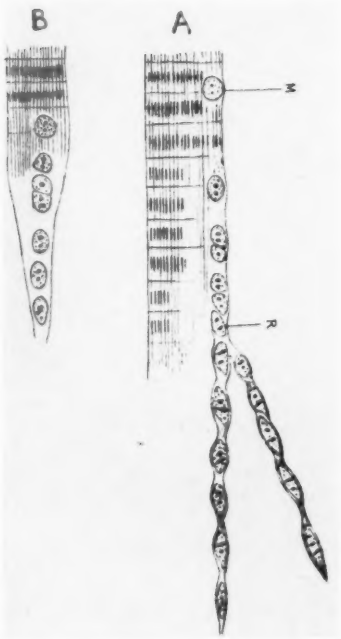

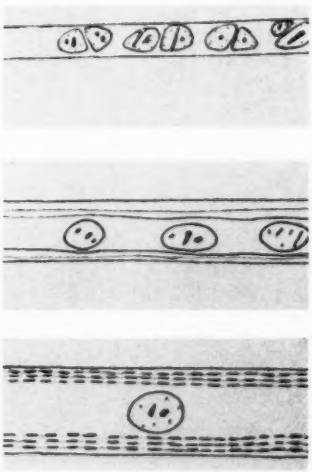

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PLATE 32

FIG. 15. Heart. Large zone of myocardial necrosis at apex of left ventricle. Conn.-5, third day of signs. Hematoxylin and eosin stain. × 300.

FIG. 16. Brain. Rarefaction following necrosis in cerebrum. Conn.-5, third day of signs. Hematoxylin and eosin stain. × 250.

FIG. 17. Brain. Encephalitis, showing marked perivascular cuffing and leptomeningitis. Conn.-5, third day of signs. Hematoxylin eosinate-azure stain. × 340.

FIG. 18. Fat. Acute necrotizing steatitis, illustrating the selective destruction of the peripheral parts of the lobules (shown as paler margins) and preservation of the central parts (shown as dark because fuchsinophilic). Interlobular edema and moderate inflammatory reaction. Conn.-5, first day of signs. Millar stain.[9] × 125.

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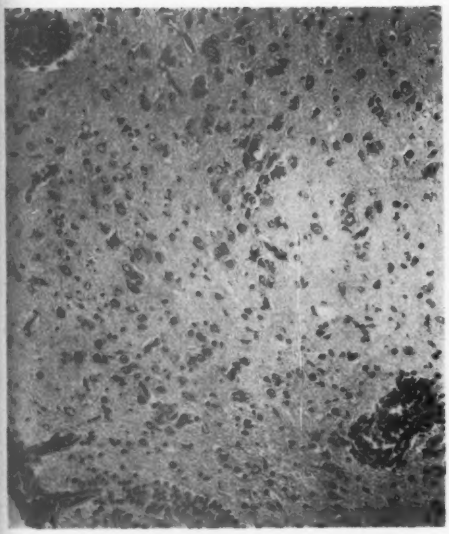

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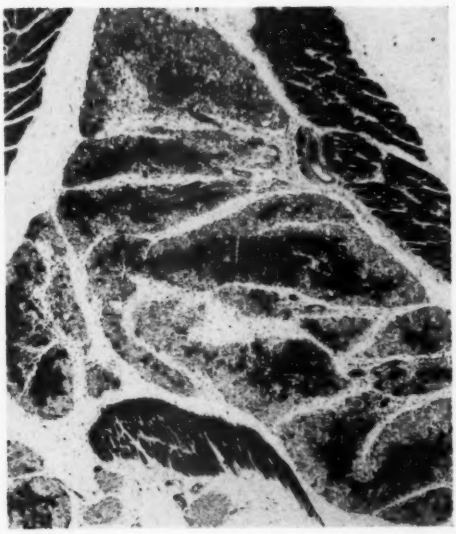

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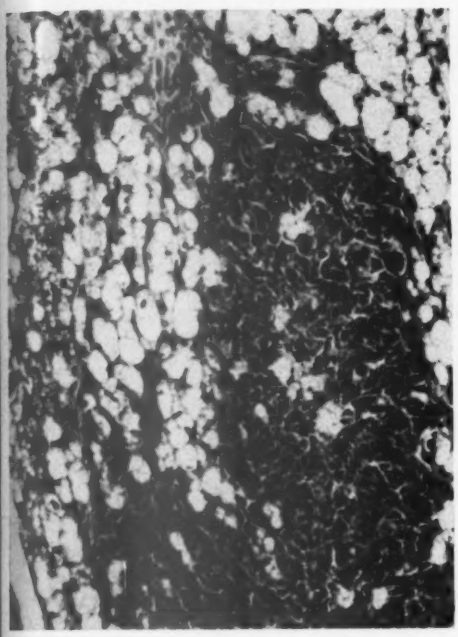
PLATE 33

FIG. 19. Fat. Granuloma formation in affected fat lobule, at least 4 days after acute injury. Ohio-1, fourth day of signs. Phloxine and methylene blue stain. × 415.

FIG. 20. Fat. Mineralization in necrotic zones of fat lobule. Conn.-5, second day of signs. Hematoxylin and eosin stain. × 185.

FIG. 21. Pancreas. Acute pancreatic necrosis, showing acinar remnants, marked interstitial edema, and moderate cellular inflammatory reaction. Conn.-5, first day of signs. Hematoxylin and eosin stain. × 400.

FIG. 22. Liver. Acute necrosis of liver cells and disorganization of liver architecture. Karyorrhexis, pyknosis, and the formation of hyaline anuclear bodies are shown. There is a moderate histiocytic reaction. Conn.-5, first day of signs. Hematoxylin and eosin stain. × 400.



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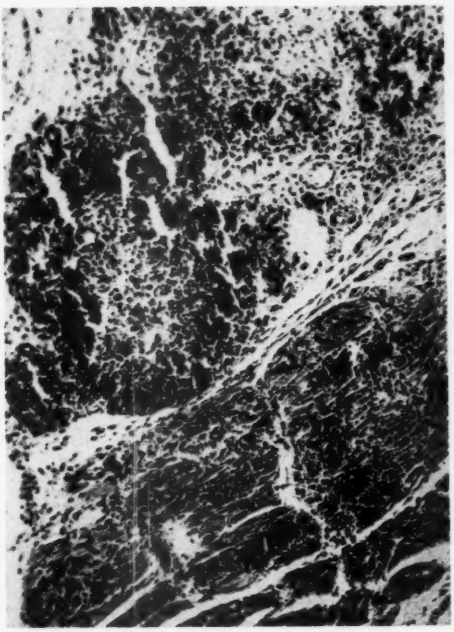

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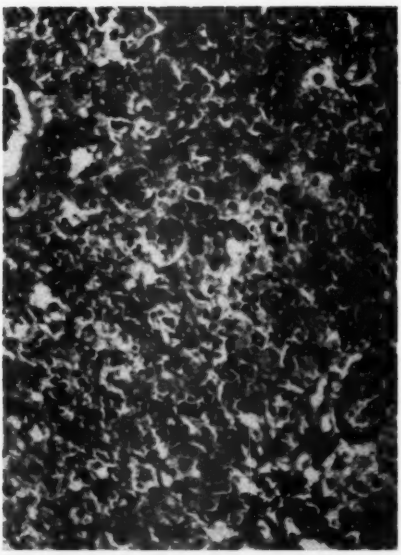

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LIVER DAMAGE IN ULCERATIVE COLITIS*

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Little is known about the occurrence of liver damage in so-called non-specific ulcerative colitis, and the scant information available reveals widely divergent opinions. Our experience has shown that it is of both academic and practical importance to know the nature and frequency of liver disease associated with ulcerative colitis. Should a causative relationship exist, should liver damage occur with significant frequency, the management of ulcerative colitis and particularly resort to intestinal surgery should be guided by this knowledge. We have therefore undertaken a review of the literature and shall present a statistical analysis of our own material and that of the Armed Forces Institute of Pathology. This study reveals that various types of liver damage occur in ulcerative colitis with a frequency higher than expected from previous reports.

REVIEW OF THE LITERATURE

Most reviews concerning the pathogenesis and etiology of hepatic cirrhosis and hepatitis have failed to refer to ulcerative colitis as a possible causative condition (Rössle,[1] Karsner,[2] and Moon[3]). Likewise, discussions of various complications of ulcerative colitis commonly have ignored liver damage (Jankelson, McClure, and Sweetsir[4]), although the occurrence of cirrhosis and of frequent fatty changes was mentioned by Monaghan.[5] In a recent monograph, Bargen[6] referred to a variety of hepatic lesions in thrombo-ulcerative colitis but did not give detailed account of his observations except for the relative frequency of fatty changes. His series was concerned only with ulcerative colitis of streptococcal origin, not including the "non-specific" type. We failed to make this distinction.

Ricketts and Palmer,[7] in a clinical study of complications of ulcerative colitis, reported the occurrence of hepatitis in 0.48 per cent, but their publication does not indicate that specific examinations of liver function were undertaken systematically in their series.

An editorial in the British Medical Journal in 1949[8] stated bluntly, "The attempt to relate the cirrhosis or other hepatic lesion to the coexisting colitis is most unconvincing."

Cirrhosis of the liver with ulcerative colitis was first described by Bargen[9] in 1929, when he reported a case proved by necropsy in a

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male, 15 years old. Subsequently, he[10] presented an additional case "apparently secondary to thrombosis of hepatic veins" in a female, 33 years of age, with a history of ulcerative colitis of 3 years' duration. In 1947 Tumen, Monaghan, and Jobb[11] published 5 instances of hepatic cirrhosis with ulcerative colitis, although one was a chronic alcoholic patient. An additional case was described by Ross and Swarts[12] as hepatic cirrhosis and necrosis with evidence of regeneration. Their second case cannot be fully evaluated since no histologic examination was made and the description indicates biliary obstruction, possibly unrelated to ulcerative colitis.

In 1949 Mallory[13] presented 2 cases of hepatic cirrhosis in association with ulcerative colitis which he interpreted as being of the postnecrotic type. Warren and Sommers,[14] in the same year, found cirrhosis in 3 of 60 necropsy cases of ulcerative colitis. Two of these were classified as toxic, the third as cirrhosis from fatty changes.

We have not found more than 11 case reports of hepatic cirrhosis associated with chronic ulcerative colitis in the literature.

Apparently, fatty livers are found very frequently in patients with ulcerative colitis. Mallory[13] spoke of 80 per cent with massive fatty infiltration in the liver among cases of ulcerative colitis. Warren and Sommers[14] found it in approximately 55 per cent, and Ross and Swarts[12] in 41 per cent. Even though the degree of fatty change may be evaluated differently by individual observers, it seems to be certain that severe fatty changes occur with significant frequency.

Other changes in the liver, however, have been mentioned only occasionally. A single case of multiple abscesses was presented by Lansbury and Bargen.[15] Two instances of so-called toxic hepatitis, described as perilobular chronic inflammatory infiltration with fibrosis and disorganization of lobular architecture, were reported by Warren and Sommers.[14] The cases of toxic hepatitis mentioned by Ross and Swarts[12] probably were the same as those reported by Warren and Sommers. The latter also observed small foci of necrosis with inflammation and infiltration in 6 cases. An isolated report of chronic hepatitis with recurrent necrosis (Harrell and McBryde[16]) closes the list of references specifically concerned with this subject.

That the accumulated tabulation of cases is incomplete can be demonstrated by an article on amyloidosis (Thompson and Rice[17]) in which the necropsy report on a male, 27 years old, mentioned parenthetically, "there was also periportal hepatic cirrhosis and ulcerative colitis." The degenerative and inflammatory lesions of the liver which Stewart and Jones[18] observed in rats suffering from chronic ulcerative cecitis may possibly be added to the findings in humans.

Purely clinical observations are of fundamental importance, since they demonstrate that ulcerative colitis may be associated with transient hepatic changes. Comfort, Bargen, and Morlock,[19] in 1938, reported 4 cases of hepatic insufficiency in which the symptoms and signs of liver disease were accentuated concomitantly with bouts of diarrhea. In one of these cases the liver disease apparently preceded the intestinal symptoms. McCannel[20] reported a similar observation, a case in which bromsulfalein retention was associated with exacerbations of chronic ulcerative colitis. Two cases of hepatic insufficiency in chronic ulcerative colitis have been reported by Johnson,[21] in only one of which improvement of both hepatic involvement and colitis was achieved. The second patient showed no improvement of either condition.

The only systematic study of liver function in ulcerative colitis which we have been able to find is that of Ross and Swarts,[12] who examined 20 patients with ulcerative colitis with the aid of various liver function tests. They came to the conclusion that there was no greater incidence of hepatic insufficiency than occurs in other maladies when uncorrected factors of severe anemia, weight loss, and/or negative nitrogen balance appear. They noticed a definite trend toward hepatic insufficiency, manifested mainly by low serum protein levels with reversal of the albumin-globulin ratio, when extensive ulcerative colitis was associated with weight loss and anemia. Hypo-albuminemia was found also in 5 cases of hepatic cirrhosis in ulcerative colitis.[11]

Material

Our own analysis is based upon a small series of 12 cases from the Charlotte Memorial Hospital and a large series which was kindly put at our disposal by the Armed Forces Institute of Pathology. From the total number of available cases of ulcerative colitis we eliminated all instances in which specific etiology was known. This included a variety of parasitic and bacterial diseases, heavy metal poisoning, uremia, and other miscellaneous conditions. There remained 81 instances of ulcerative colitis of unknown etiology from the material of the Armed Forces Institute of Pathology, which we have included in our total of 93 cases.

Findings

Our study has revealed various hepatic lesions with ulcerative colitis, differing greatly in nature and extent. We shall divide the presentation of our findings into groups of degenerative and inflammatory lesions, describing the salient points of each type to which our interpretations are added. Each group will be illustrated by one or more

case reports. In the choice of these, cases in which significant clinical observations were available were given preference.

DISSOCIATION AND NECROSIS

Dissociation and necrosis were found in 8 cases, or 8.6 per cent. Only 2 cases of advanced dissociation of liver cell cords were encountered in which there was neither necrosis nor inflammatory reaction (cases A-163 and A-917). In portions of the sections it could be recognized that the process of disintegration had begun in the center of hepatic lobules, a narrow peripheral margin still being intact (Figs. 1 and 2). There was no evidence of passive congestion. In a great number of cases central dissociation was observed, but not included in the tabulation because of the doubtful significance of this finding. The diffuse dissociation in the 2 cases mentioned was so conspicuous that we had to take cognizance of this lesion although there was no evidence of regeneration.

Six cases of focal necrosis were observed. In 2 of them there was found inflammatory reaction about foci of necrosis in the form of infiltration with polymorphonuclear leukocytes (A-242 and A.F.I.P. Acc. 139406).* The inflammation varied in intensity and some of the foci in the same liver showed no inflammatory reaction.

In 2 cases (A.F.I.P. Acc. 153167 and 96518) numerous bile pigmented macrophages could be found within the areas of necrosis and many of the detached and partially disintegrated liver cells were loaded with bile pigment (Fig. 4). Two of the cases revealed large areas of necrosis (Fig. 5) without any reaction (A-57 and A.F.I.P. Acc. 167759) except for a zone of hyperemia in one of them (A.F.I.P. Acc. 167759). The changes were so conspicuous that they deserve notice. In one instance the foci were so large as to become confluent in many areas. In all of these cases the necrotic foci frequently were found in an eccentric position, often in juxtaposition to the central vein.

The question has been raised as to whether the dissociation of liver cells and central necrosis should not be regarded as agonal processes. Evidence presented by Popper[22] is indeed convincing. However, it is difficult to conceive that the severe lesions to which we have reference could be a post-mortem product. If they result from agonal destruction, we must assume that damage to liver cells prior to death predisposes to the change observed at necropsy. Without evaluating the significance of this process at this time, we register the occurrence of

* The cases from the Armed Forces Institute of Pathology are cited according to the accession number (Acc.).

severe dissociation and necrosis in 8 cases, of which 5 showed reaction of mesenchymal tissue, while 3 were without reaction.

Illustrative Cases

M. W. (A–242) was a white male, 67 years old, who was admitted to the Charlotte Memorial Hospital on April 11, 1943, complaining of vertigo, dyspnea, and tarry stools. On admission the red blood cell count was 1,750,000; the white blood cell count was normal. The prothrombin time was 56 per cent of normal. In spite of blood transfusions and other supportive measures, the patient failed to respond and died 4 days after admission, on April 15, 1943. The clinical diagnosis was acute ulcerative colitis.

At necropsy there was an acute ulcerative colitis of the sigmoid colon. The liver weighed 1650 gm. and was normal grossly. Histologically, there were diffusely distributed foci of necrosis involving the centers of nearly all lobules (Fig. 3). The parenchymatous elements were markedly disintegrated, many of the cells being small, relatively dark-staining, and partially fragmented. The nuclei were pyknotic and fragmented. Other cells within the centers of lobules, and often in direct contiguity with those described, were rather large and possessed a foamy cytoplasm, which did not contain fat, nor did it give a positive glycogen reaction with periodic acid stain. Some of these cells had undergone the same disintegration as had the cells in the centers of the lobules. There was an associated moderate inflammatory infiltration composed of polymorphonuclear leukocytes within the zones of necrosis, but this was not encountered in all necrotic areas. The reticulum was found to be collapsed within the necrotic areas. The latter were most often eccentrically located, although they occasionally completely encircled the central veins. There were diffusely distributed foci of marked inflammatory infiltration composed of lymphocytes, plasma cells, polymorphonuclear leukocytes, and infrequently eosinophils in the periportal spaces. However, all triads were not involved.

Impression. Central and eccentric necrosis with little inflammatory reaction.

F. H. (A.F.I.P. Acc. 96518), a white male, 27 years of age, was admitted to an army general hospital on February 23, 1943, with a history of annual recurrent attacks of mild diarrhea for 7 years. A persistent severe bloody diarrhea of 1 month's duration led to his admission. Physical examination revealed evidence of marked dehydration, moderate abdominal distention, and a palpable liver 1 fingerbreadth below the right costal margin. There was slight tenderness in the right lower quadrant of the abdomen. On admission the red blood cell count was 2.54 millions per cmm.; hemoglobin was 50 per cent; the white blood cell count was 4,250 per cmm. Repeated blood cultures were negative. Repeated stool examinations were negative for ova, parasites, and pathogenic organisms. Routine agglutinations were normal. Despite therapeutic measures, the diarrhea increased in

severity, ranging from ten to thirty movements daily. Because of his poor condition, surgical treatment was not attempted and he died on April 24, 1943. The clinical diagnosis was chronic ulcerative colitis, mild, intermittent, with acute fulminating exacerbation.

At necropsy a severe chronic ulcerative colitis was demonstrated. Histologically, the liver architecture was preserved but somewhat obscured by large foci of central necrosis which occasionally were confluent. The necrotic foci were sharply delineated and there was no inflammatory reaction within or around them. They comprised one-third to two-thirds of the hepatic lobule. Within the areas of necrosis were found pigmented macrophages and increased amounts of pigment in some of the necrotic liver cells proper. The interlobular inflammatory infiltration was insignificant.

Impression. Extensive central necrosis without inflammatory reaction.

FATTY CHANGES

Fatty changes were studied from 14 cases, or 15 per cent: A-20, A-796, A.F.I.P. Acc. 217561, 239546, 75963, 151307, 204696, 230037, 33244, 38883, 57932, 59597, 89867, 106603. Two additional cases were excluded because the fatty changes in the liver could be related to conditions other than ulcerative colitis: one patient was known to be alcoholic (Acc. 158198); the other, a child (Acc. 84159), showed findings in the pancreas compatible with early cystic fibrosis.

The relative infrequency with which we have encountered significant degrees of fatty changes has been surprising to us. The great variation in previous reports, ranging from 40 to 80 per cent, seems to indicate that subjective interpretation of the degree of fatty infiltration plays an important part in the evaluation of the findings. Our own evaluation of 15 per cent is probably explained by the fact that only severe involvement was tabulated. We have gained the impression that in most of the lesser degrees, the fatty infiltration was either confined to, or was by far more prominent in, the periphery of the lobules.

It may be noteworthy that the liver cells in these cases frequently demonstrated evidence of accelerated regeneration. In some of them the nuclear changes were conspicuous, but definite correlation with the course or duration of the colitis could not be established.

Illustrative Case

A. W. (A.F.I.P. Acc. 204696) was a white male, 20 years of age, who was transferred to the Walter Reed General Hospital on August 4, 1947, from another army hospital because of a progressively severe bloody diarrhea of approximately 3 months' duration. Repeated bacteriologic and serologic examinations disclosed no specific etiologic agent. Various antibiotics, as well as emetine, had been ineffective. At admission he was acutely ill, dehydrated, and underweight. There was general-

ized abdominal tenderness as well as hyperactive peristalsis. Admission blood counts were normal. Subsequently, the total serum proteins were 5.2 gm. with 2.5 gm. of albumin per 100 cc. The prothrombin time was 19 seconds with a control of 13 seconds. A barium enema revealed extensive changes throughout the entire colon, as well as the terminal ileum, suggestive of ulcerative colitis. Since conservative therapeutic measures were to no avail, an ileostomy was performed on September 17, despite which the diarrhea became more severe. The icterus index on September 29 was 18. The patient had massive rectal hemorrhages, and died on October 5, 1947. The clinical diagnosis was subacute continuous fulminating ulcerative colitis.

At necropsy there was a non-specific ulcerative colitis. Except for severe, diffuse, fatty metamorphosis, the liver was not remarkable.

METALLAXIS OF LIVER PARENCHYMA

Some degree of metallaxis of liver parenchyma was found in 3 cases, or 3.2 per cent. In only one case (A-1089) was definite cirrhosis observed. It was associated with moderate fatty changes, moderate interlobular fibrosis, and bile duct proliferation. Periportal round cell infiltration was insignificant. There can be no doubt that in this instance the replacement of normal liver tissue by pseudolobules constituted the fundamental pathologic process, which was out of proportion to the relatively insignificant changes in the periportal areas.

In 2 additional cases similar lesions were noted but to a much lesser degree (A-15 and A-423). Some lobules still showed normal architecture, but in many the configuration and the radiating arrangement of liver cell cords was obscured (Figs. 7 and 8). The central veins were either displaced or could not be found. In neither of these instances were there significant changes in the periportal tissue, but in both were found areas of fibrosis in centrolobular position immediately next to the walls of central veins.

It can be stated that the liver changes in these 3 cases were not due to inflammatory lesions, but in all probability were related to metabolic disturbances. The irregular distribution of metallaxis and central lobular fibrosis cannot be held against such a supposition. The formation of pseudolobules, particularly in early phases, must not be expected to be necessarily uniform in distribution. A tendency to at least potentially impaired liver function associated with hypoproteinemia may be assumed in a relatively large number of cases of severe ulcerative colitis (Ross and Swarts[12]) in which the organ is at the threshold of protein deficiency. Since protein deficiency, among other factors, is known to result in necrosis of the parenchyma, functional variation in intralobular blood supply may account for topographic variations in the severity of its effect.

Centrolobular fibrosis is not commonly associated with pseudolobulation or hepatic cirrhosis in man other than in cases of chronic passive congestion. Experimentally, however, this form of hepatic cirrhosis has been produced in dogs under various conditions related to dietary and other factors (Gillman and Chaikoff[23]) and therefore it appears permissible to associate the central fibrosis in our 2 cases with the metallaxis of the hepatic parenchyma. We have therefore interpreted these 3 cases as liver damage based on metabolic disturbances, 2 of them showing changes only in a more focal distribution in an early stage, one of them in the form of fully developed cirrhosis.

Illustrative Cases

J. B. (A–423), a white male, 60 years old, was admitted to the Charlotte Memorial Hospital on September 23, 1944, with a history of recurring attacks of bloody diarrhea for a period of 15 to 20 years. On admission the liver was enlarged 2 fingersbreadth below the right costal margin. The red blood cell count was 2,610,000 per cmm. with 7.4 gm. of hemoglobin. The white blood cell count was normal. A roentgenologic examination of the lower bowel revealed changes typical of chronic non-specific ulcerative colitis. During his third hospital week he began to have repeated hemorrhages from the lower bowel, and subsequently expired on October 23, 1944. The clinical diagnosis was chronic ulcerative colitis, intermittent, with a severe fulminating exacerbation.

At necropsy the entire colon showed typical changes of severe chronic diffuse ulcerative colitis. The liver weighed 1700 gm. The surface was finely granular. The cut surface showed accentuation of lobulation. The extrahepatic bile ducts were somewhat distended, but were patent throughout.

Histologically, the lobular architecture was obscured (Figs. 9 and 10). The central vein could be found only occasionally. There was a rather marked, predominantly periportal, fatty change of parenchymatous cells, apparently accounting for the accentuated lobulation noticed grossly. In some lobules there was marked fibrosis around the central veins. The periportal spaces showed only insignificant round cell infiltration and no fibrosis. The bile ducts were not involved.

Impression. Pseudolobulation with occasional areas of central fibrosis. The findings were not compatible with biliary cirrhosis, which was taken into consideration because of dilatation of extrahepatic bile ducts.

J. R. (A–1089) was a white male, 19 years old, who was admitted to the Charlotte Memorial Hospital because of a recurrence of bloody diarrhea for 4 days prior to admission. There was a history of periodic attacks of bloody diarrhea of variable intensity, generally severe, occurring every 2 to 3 months over the previous 6 years. A barium enema showed changes characteristic of chronic ulcerative colitis. On admission the abdomen was distended, tympanitic, and tender throughout. The red blood cell count was 3,890,000 per cmm. with 11 gm. of hemoglobin.

The total leukocyte count and differential count were normal. The total serum proteins were 6.2 gm. per 100 cc. Following blood transfusions, he was subjected to a partial colectomy on November 2, 1948. On the following day he was deeply jaundiced, with an icterus index of 88. After repeated severe rectal hemorrhages, he expired on November 8, 1948, on his sixth postoperative day. The surgically removed specimen showed changes typical of severe chronic ulcerative colitis as did that portion of colon remaining at necropsy. The clinical diagnosis was chronic ulcerative colitis, with acute exacerbation.*

The liver was enlarged, weighing 2,245 gm. The capsular surface was diffusely finely granular and tan-yellow. On dissection it was likewise finely and diffusely granular, possessing nodules of variable size, the largest of which measured 0.2 cm. in diameter. These nodules were separated by fine, grayish white, interlacing bands of rather firm fibrous tissue. The inferior margins of the liver were somewhat rounded.

Histologically, pseudolobulation was a conspicuous feature (Fig. 6). In addition, there were areas of irregularly distributed, rather marked dissociation of liver cell cords. Moreover, many of these cords were markedly distorted, mainly in the vicinity of the areas in which parenchymatous cells showed mild fatty changes with subsequent disintegration and actual necrosis without inflammatory reaction. The disintegration occurred mainly in mid-zonal areas with associated collapse of the reticulum. Occasionally encountered were bile casts, predominantly near the areas of degenerative changes.

Impression. Cirrhosis with small foci of necrosis.

BILE CASTS

Bile casts were found in only 2 cases, or 2.2 per cent: A.F.I.P. Acc. 190983 and 108270. In spite of the paucity of morphologic changes in the liver parenchyma, the occurrence of numerous bile casts appears to be significant. In one case casts were found in a few bile ducts. In neither instance could their presence be explained by obstruction of the bile system. The morphologic observations do not offer tangible evidence for their pathogenesis, but their presence indicates indisputable evidence of functional liver damage at the time of death or in the recent past.

Illustrative Case

S. Z. (A.F.I.P. Acc. 190983) was a white male, 23 years old, who was admitted to the Walter Reed General Hospital on October 21, 1946, with a history of recurrent short bouts of diarrhea in 1942 and again in 1945. In June, 1946, diarrhea became progressively more severe and subsequently bloody, despite treatment. On September 25, 1946, an ileostomy was performed with no permanent alleviation of

* In reviewing the literature on this subject, it occurred to us that this case might have been identical with one reported by Johnson.[21] This was verified by personal communication.

symptoms. Following subsequent massive hemorrhages, he was transferred to the Walter Reed Hospital where his red blood cell count was 2,900,000 per cmm. with hemoglobin 9.7 gm. The white blood cell count was normal. The serum bilirubin was 3.6 mg. per cent. Bacteriologic studies were negative. Despite antibiotics and blood transfusions he maintained a spiking daily fever, continued to bleed rectally, and subsequently expired on November 4, 1946. The clinical diagnosis was subacute fulminating ulcerative colitis.

At necropsy a severe chronic ulcerative colitis was found. Histologic sections through the liver showed well preserved architecture. There was a mild peripheral lobular fatty change and some evidence of regeneration. The conspicuous feature was the presence of numerous, irregularly distributed bile casts in bile capillaries and occasionally in small bile ducts (Fig. 11). Histologic sections and the gross protocol gave no explanation for bile stasis.

Impression. Intrahepatic bile stasis.

SMALL FOCI OF INFLAMMATION

Small foci of inflammation were found in 5 cases, or 5.4 per cent: A.F.I.P. Acc. 217561, 239546, 236758, 271764, 75963. These may occur anywhere within the lobule or in the periportal tissue. Those occurring in the parenchyma proper were associated with necrosis of liver cells, but the inflammatory infiltrations seemed to be the predominating feature. They resembled closely the lesion illustrated by Warren and Sommers,[14] but designated by them as focal necrosis. They also appeared similar to parenchymatous changes in cases of severe interlobular hepatitis in which the inflammatory process extended into the lobule. We have therefore interpreted these foci as probably transitory, diminutive lesions, analogous in other respects than size to the massive changes designated as interlobular hepatitis. Even though these foci are small, scattered, and infrequently observed, they may serve as evidence of transitory embolic involvement of the liver in ulcerative colitis. Phlebitis of small intrahepatic portal branches illustrates this assumption.

Illustrative Case

M. C. (A.F.I.P. Acc. 236758) was a white male, 52 years of age, who became ill suddenly on June 7, 1948, with severe abdominal pain. An exploratory laparotomy is said to have shown gangrenous areas in the descending colon. A loop colostomy was performed. On August 12, 1948, he developed diarrhea with four to six movements daily, most of which contained pus. On August 27, he was admitted to the Lawson Veterans Administration Hospital, at which time he was extremely emaciated and dehydrated. The abdomen was moderately distended with fluid, and the enterostomy opening drained loose, mucoid material. The hemoglobin was 14 gm. The leukocyte count was normal. The total serum proteins were 5.3 gm. per cent. The cephalin flocculation was 3 plus at 48 hours; thymol turbidity,

4.2 units; serum bilirubin, 2.3 mg. per cent. He expired on September 10, 1948. The clinical diagnosis was subacute fulminating ulcerative colitis.

At necropsy there was an ulcerative colitis. Histologic sections through the liver showed a small and discrete focus of polymorphonuclear leukocytic infiltration within the wall of a central vein, extending somewhat into the adjacent connective tissue, and in an irregular manner into the adjacent liver tissue proper (Figs. 12 and 13). There was a moderate passive congestion and there were regenerative changes in liver cells, particularly next to foci of central dissociation.

Impression. Focus of inflammatory infiltration of central vein, and adjacent liver tissue (may be forerunner of central fibrosis?).

INTERLOBULAR HEPATITIS

Nine cases, or 9.7 per cent, showed interlobular hepatitis: A.F.I.P. Acc. 277125, 78852, 141078, 149528, 211619, 212899, 269837, 106603, and A-860. A tenth case was omitted (Acc. 111785) because of the possibility that the interlobular hepatitis may have been cholangitic, secondary to marked chronic cholecystitis. The lesion consisted of marked inflammatory infiltration of the interlobular connective tissue with a variety of round cells, including lymphocytes, polymorphonuclear leukocytes, plasma cells, and macrophages (Figs. 16, 17, 18, 22, 23, and 24). It appears that the lobular architecture is at first well preserved. In many instances the inflammatory infiltration encroaches upon adjacent parenchyma with necrosis of liver cells. In later phases, bile duct proliferation is recognizable and finally pseudolobulation may set in. The latter is irregularly distributed, meaning that in some areas the lobules are well preserved and in others, particularly where the interlobular inflammation is most marked, they become irregular in shape and size. Their central veins are displaced or are not recognizable.

The irregularity with which the inflammatory process is spread throughout the section is a remarkable feature. In one case so little infiltration was seen in one section that it would not have been classified as interlobular hepatitis except that large massive patches were encountered in an additional block of tissue. We feel, therefore, that more cases of interlobular hepatitis possibly would have been registered if more tissue had been available. It is likewise difficult to determine how much interlobular infiltration should be looked upon as physiologic and when to classify the lesion as pathologic. We realize that this, to some extent, is left to the experience of the observer, but for this study we have included only cases which left no doubt concerning the severity of the process.

In later phases the infiltration may subside somewhat, but fibrosis and bile duct proliferation remain (A-860).

We interpret this process tentatively as a reaction to "toxins" or bacteria which invade the liver by the portal system. In favor of such an assumption is the occasional portal phlebitis, the lack of parenchymatous involvement in the earlier phases or lesser degrees, and the fact that the biliary tree is not involved.

Illustrative Cases

J. B. (A.F.I.P. Acc. 149528), a Hungarian white male, 45 years old, was an inmate of the Dachau Concentration Camp. He was admitted to the 127th Evacuation Hospital on May 6, 1945, for incision of a large fluctuant abscess overlying the right scapula. Prior to admission the patient had been treated for enterocolitis which had developed apparently following typhus fever. On May 10, he developed severe diarrhea with seventeen liquid, blood-tinged stools daily. He expired on May 18, 1945. This case could not be classified because of inconclusive history.

At necropsy there was severe ulcerative colitis. Histologically, the lobular architecture of the liver was partially preserved. Occasional pseudolobules were found. Fatty changes were absent and degenerative changes of liver cells were found only in the immediate vicinity of inflammatory changes. The outstanding feature was a heavy inflammatory infiltration (Figs. 19, 20, and 21) composed mainly of lymphocytes with occasional polymorphonuclear leukocytes and eosinophils in the interlobular connective tissue, extending, in an ill defined fashion, deep into the hepatic lobules. These latter areas showed a much richer infiltration of polymorphonuclear leukocytes. In many of the lobules, the walls of the branches of the portal vein were diffusely and heavily infiltrated by similar inflammatory cells, and in some areas, portions of the vein were completely replaced by such an infiltration. Also there were occasional portal triangles with fibrosis as well as marked bile duct proliferation. The changes were widespread, but irregularly distributed. Bile ducts were not involved, and there was found no evidence of bile stasis.

Impression. Severe interlobular hepatitis.

H. M. (A.F.I.P. Acc. 277125) was a white male, 69 years of age, who was first admitted to the Veterans Administration Hospital, Bay Pines, Florida, in February, 1947, with bloody diarrhea. Following that admission he did fairly well for 2 years when, after severe diarrhea of from twelve to fourteen movements daily for a period of 1 month, he was readmitted on July 29, 1949. On admission there was evidence of dehydration. The liver border was palpable, firm, hard, and at the right costal margin. The red blood cell count was 5,280,000, with hemoglobin 77 per cent. The white blood cell count was 12,900. Repeated stool examinations were negative. Cephalin flocculation was 4 plus at 48 hours and the thymol turbidity was 10 units. The serum bilirubin was 0.89 mg per cent. Wassermann and

Kahn reactions on the blood were positive. Barium enema revealed a severe ulcerative colitis involving the entire colon. Despite treatment, he became severely anemic, and expired on August 15, 1949. The clinical diagnosis was chronic intermittent ulcerative colitis, with an acute protracted fulminating exacerbation.

At necropsy severe ulcerative colitis, involving the entire colon, was encountered. Histologically, the lobular architecture of the liver was preserved, although some central dissociation with increased pigmentation of liver cells was seen. There were found numerous extensive foci of necrosis of liver cells with very heavy inflammatory infiltration, mainly polymorphonuclear leukocytes and lymphocytes (Figs. 14 and 15). The foci were large and situated in either the mid-zone or the periphery of lobules. They were often ill defined, with a great number of polymorphonuclear leukocytes, infiltrating irregularly the adjacent portions of hepatic lobules. Infiltration by polymorphonuclear leukocytes was found in such areas in which the liver cell necrosis was not clearly demonstrable. With this process were large collections of pigmented macrophages in some, but not all of the foci of necrosis. The periportal spaces were moderately infiltrated, predominantly with lymphocytes, but there was no fibrosis. There was no bile duct involvement and no evidence of bile stasis. The distribution of inflammatory foci was irregular but diffuse.

Impression. Acute interlobular hepatitis with necrosis. The impression was gained that inflammatory infiltration was so preponderant that it could not be regarded merely as reaction to liver cell necrosis. The latter might, in fact, be secondary to the inflammation.

Discussion

Relation of colitis to liver damage

Of the 93 cases analyzed, pathologic conditions were observed in the livers of 37 (40 per cent). If one assumes that interlobular hepatitis, pseudolobulation, cirrhosis, multiple bile casts, and severe fatty changes are of actual or potential clinical significance, it is noteworthy that 26 such cases (28 per cent) were found in our series. In our opinion these observations indicate that the relationship between ulcerative colitis and liver damage is more than coincidental.

It becomes necessary to distinguish between two pathogenetically separate groups of liver changes, namely, those of degenerative and those of inflammatory nature. Not all of the cases can be segregated strictly in this manner since some of them showed lesions of both types. It is possible, however, to summarize the cases in a table presenting the preponderant type of involvement.

Since quantitative estimation of degree of severity enters into the

evaluation of findings and may thus affect the statistical results, we reviewed 1000 necropsies, applying the same criteria used in the cases of colitis. From the control series were excluded all cases with liver changes attributable to known specific causes, such as inflammatory lesions of the bile system, septicemia, exogenous toxins, vascular lesions, trauma, and also all premature and newborn infants. The following table indicates the relative frequency of liver damage in the control series compared to that of ulcerative colitis.

	Ulcerative colitis 93 cases		Control series of 1000 cases
	Number	Per Cent	Per Cent
Degenerative lesions			
Cirrhosis	1	1.08	2.3
Pseudolobulation	2	2.15	0.8
Multiple bile casts	2	2.15	0.4
Necrosis and dissociation	8	8.60	6.6
Fatty changes	14	15.05	1.4
	27	29.03	11.5
Inflammatory lesions			
Interlobular hepatitis	9	9.68	1.1
Foci of inflammation	5	5.38	1.0
	14	15.06	2.1

It is apparent that fatty changes and inflammatory lesions occur with much greater frequency than would be expected if their relationship to ulcerative colitis were purely coincidental. The degree of fatty infiltration designated as "severe" was observed more than ten times as frequently in the colitis group than in the control series. The type and severity of inflammation, designated as interlobular hepatitis, occurred nine times as frequently, and small inflammatory foci were seen more than five times as often as in unselected cases. It is likewise apparent that diffuse changes of the hepatic parenchyma, manifested by metallaxis with formation of atypical lobules (pseudolobulation) and by the presence of numerous bile casts, are significant since these "degenerative" lesions are found two and one-half and five times, respectively, as often as in the control series. Cirrhosis seems to be less common in cases with ulcerative colitis, but these figures are misleading because the age distribution was not taken into consideration. In the light of the other observations, particularly the frequency of fatty changes, even a single instance at the age of 19 among 93 cases is of significance. Zonal necrosis and severe degrees of dissociation were not observed more often in cases of ulcerative colitis than in the control series.

In evaluating the frequency with which ulcerative colitis is asso-

ciated with liver damage, it is necessary to consider that liver involvement may be transitory and, therefore, not be found at the time of death. Bargen's[9] original observation and those of others are important in this respect. These authors observed accentuation of clinical signs and symptoms of liver disease during bouts of diarrhea. The frequency, therefore, with which reversible changes, such as necrosis and fatty change, are observed at necropsy may depend upon the presence of fleeting attacks of liver involvement at the time of death. Only when colitis is continuous, fulminating, and unabated, may permanent liver damage be expected. All of our cases of interlobular hepatitis and the one instance of cirrhosis fell into this category. The reverse, however, does not hold true. Not all cases of continuous fulminating colitis showed liver damage. One may quite reasonably ask why liver damage is not found clinically or anatomically with greater frequency than 40 per cent. We have already implied a partial answer by pointing out that the most common remittent form of chronic ulcerative colitis gives the liver sufficient time for repair during the period of remission.

The study of our cases and those from the literature has given us ample reason to postulate that both degenerative and inflammatory lesions of the liver are direct results of ulcerative colitis.

DEGENERATIVE CHANGES

Fatty changes, necrosis, and cirrhosis are known to result from nutritional deficiencies. There is experimental evidence that diets deficient in protein can produce acute hepatic necrosis. Fatty changes in the liver and diffuse fibrosis likewise have been produced in a similar manner (Himsworth and Glynn[24] and others[23,25]). Ulcerative colitis is associated with inadequate intake of food, faulty utilization, and poor absorption, probably connected with the rapid transit of intestinal content. According to Jankelson and McClure,[26] hypoproteinemia with a normal albumin-globulin ratio develops in 55 per cent of the cases of ulcerative colitis.

The tabulation of our cases reveals an additional observation which seems to corroborate Himsworth and Glynn's[24] contention that fatty infiltration serves as protection against necrosis. As mentioned previously, in many of the cases more than one type of change was observed. Among 16 cases of severe fatty changes we encountered 3 with small foci of inflammation, one with interlobular hepatitis, and one with cirrhosis. Foci of necrosis, however, or dissociation were not observed in severe fatty livers. It appears to us that most of the degenerative changes under discussion are reversible and that permanent damage in the form of cirrhosis is a rare complication of ulcerative colitis.

INFLAMMATORY CHANGES

Foci of Inflammation

There are two reasons for including the seemingly insignificant scattered foci of inflammation in our tabulation. In the first place, as pointed out previously, those foci demonstrate that a transitory invasion of the liver by either toxins or bacteria through the portal system can occur. Inflammatory lesions in the liver have been produced experimentally by injection of streptococci into the radicles of the portal system (MacMahon and Mallory[27]). In the second place, it is possible that they merely indicate a more diffuse damage to the liver tissue, which otherwise finds no significant morphologic manifestation. Of the 5 cases with foci of inflammation, 2 revealed clinical evidence of additional liver involvement. In one case an associated fatty change may have accounted for it, but in the second case (A.F.I.P. Acc. 236758) jaundice and laboratory evidence of functional hepatic deficiency could not be accounted for by histologic findings in the liver parenchyma. The foci of inflammation alone could not explain the diffuse liver damage.

Interlobular Hepatitis

Of particular interest to us were the cases designated as interlobular hepatitis. A comparison of the 9 cases in our series seems to indicate clearly that the process begins in the portal triangles, extending in the more severe instances around most of the circumference of the lobules. The process involves the parenchyma in a diffuse fashion only in its later phases, when pseudolobules are formed. The lesion described is in no way specific, but its occurrence in conjunction with ulcerative colitis seems to have escaped attention. Two cases of Warren and Sommers' series,[14] recorded as toxic cirrhosis, resemble, by description, later phases of our interlobular hepatitis. The high percentage of our series stands in contrast to previous reports. An explanation may be found in the irregular patchy distribution. Extensive lesions in one block of tissue may be absent in another.

We have chosen the purely descriptive term of interlobular hepatitis because we possess merely circumstantial evidence that the "toxic" or bacterial agent reaches the lesion by the portal system. The cases showed no evidence of generalized septicemia and no biliary obstruction except for some dilatation of the common bile duct in one instance. Microscopically, no specific relation of the inflammatory infiltration to bile ducts could be noticed. The latter were often situated within the areas of periportal inflammation, but showed no significant changes of

their walls, and did not contain polymorphonuclear leukocytes. Involvement of the wall of the portal vein branches, however, was observed. Since foci of portal phlebitis have been described in cases interpreted as primary biliary infection (MacMahon[28-30] and others), we can state only that our findings, though highly suggestive of portal origin, cannot be taken as complete proof.

It is of interest to note that interstitial pancreatitis recently has been reported in 53 per cent of cases of ulcerative colitis by Ball, Baggenstoss, and Bargen.[31] Whether pancreatitis and hepatitis are pathogenetically related cannot be stated definitely. We noticed several cases with pancreatic changes resembling cystic fibrosis. In fact, one such instance in a child with massive fatty infiltration of the liver was excluded from our series of ulcerative colitis on the assumption that the liver changes were related not to the colitis but to the pancreatic lesion.

The possibility that an interlobular inflammation may be the result of hyperergia cannot be ruled out. Even parenchymatous changes, such as focal necrosis, have been interpreted as allergic reactions and have been produced experimentally (Hartley and Lushbaugh[32]). Also, antibiotics used in cases of ulcerative colitis have been held responsible for liver damage. Our observations do not contribute substantially to the evaluation of a possible allergic factor one way or another.

Although some of the cases of ulcerative colitis showed peritonitis, it is highly improbable that the peritonitis can be held responsible for the inflammatory changes in the liver parenchyma. There exists no directly demonstrable or statistical relationship. In addition, in the 11 cases of interlobular hepatitis of the control series, only one revealed peritonitis.

With all the evidence in view, we have, at least tentatively, concluded that interlobular hepatitis of this type is vascular in origin.

No conclusive statement can be made concerning the clinical significance of this hepatic lesion. We were impressed with the contrast between the conspicuous and often extensive anatomical findings and the lack of clinical signs and symptoms. Only one of 9 cases showed jaundice and a 4-plus cephalin flocculation test. This may be explained by the fact that in the majority of instances, diffuse parenchymatous changes were not present. Clinical signs of functional impairment, however, were not noticed even in cases in which pseudolobulation could be demonstrated. A clinicopathologic correlation therefore must be deferred until specific and more complete clinical data are available in future cases.

Clinical Observations

The clinical information, as may be expected from our approach to the problem through necropsy records, was incomplete in most instances. Liver function tests were omitted in some cases in which clinical findings suggested the possibility of liver damage. One case illustrates the inadequacy of clinical examination most clearly. The necropsy (A.F.I.P. Acc. 108270) showed multiple bile casts, but the clinical records do not refer to previous or present liver involvement. The latter may have been transitory, but this experience demonstrates that liver involvement in ulcerative colitis cannot be ruled out clinically unless repeated examinations specifically directed toward determination of liver function are made. The conduct of such tests should be guided by the knowledge that evidence of liver damage may be found only during the periods of diarrhea.

Even though available records were deplorably incomplete, certain important conclusions can be drawn. In all cases analyzed, a palpably enlarged liver was mentioned 3 times: in one instance, associated with pseudolobulation (A-423); in one, with necrosis (A.F.I.P. Acc. 96518); and in one, with fatty liver and foci of inflammation (A.F.I.P. Acc. 239546). Jaundice was noted in 6 instances (A-1089, and A.F.I.P. Acc. 204696, 230037, 190983, 277125, 236758), and in 2 others (A.F.I.P. Acc. 151307 and 269837) in which it could have been the result of transfusion reaction. Insufficient data on the charts do not permit definite conclusions in this respect. There was an additional case (A.F.I.P. Acc. 217561) showing low total protein content in the blood and a 2-plus cephalin flocculation test which was not considered sufficient evidence of liver damage. Jaundice was noted in 3 cases of fatty changes, in one case of cirrhosis, in one case of hepatitis, and in one case of intrahepatic bile stasis.

It can be stated, therefore, that clinical evidence of liver involvement was present in 9 cases, that is, approximately 10 per cent (jaundice in 6.5 per cent, enlargement of liver in 3.5 per cent). We believe that these data are of significance, particularly since we have pointed out the evidence supporting our contention that specific examination along this line is likely to reveal a greater frequency of cases of impaired liver function in ulcerative colitis.

Summary

The examination of livers in 93 cases of non-specific ulcerative colitis showed various pathologic changes in 40 per cent of the cases.

The frequency of some hepatic lesions in cases of ulcerative colitis exceeds by five to ten times that in a large control series.

In our series degenerative changes occurred approximately twice as often as inflammatory lesions and with much greater frequency than previously reported. It is assumed that most of these changes are transitory.

It is likely that most degenerative changes are related to recurrent attacks of diarrhea and are metabolic in origin.

Diffuse metallaxis of liver parenchyma is not common and actual cirrhosis is rare.

Severe, irregularly distributed, interlobular hepatitis was observed in almost 10 per cent of the cases. It is assumed that this lesion is portal in origin.

Definite correlations between the morphologic changes and clinical signs and symptoms could not be established because of incomplete clinical observations. Even under these circumstances, however, 10 per cent of the cases of ulcerative colitis revealed clinical evidence of liver involvement.

We wish to express our sincere appreciation to the Armed Forces Institute of Pathology for making the material available for this study.

REFERENCES

1. Rössle, R. Entzündungen der Leber. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1930, **5**, Pt. 1, 243–505.
2. Karsner, H. T. Morphology and pathogenesis of hepatic cirrhosis. *Am. J. Clin. Path.*, 1943, **13**, 569–606.
3. Moon, V. H. Experimental cirrhosis in relation to human cirrhosis. *Arch. Path.*, 1934, **18**, 381–424.
4. Jankelson, I. R., McClure, C. W., and Sweetsir, F. N. Chronic ulcerative colitis II: complications outside the digestive tract. *Rev. Gastroenterol.*, 1942, **9**, 99–104.
5. Monaghan, J. F. Ulcerative Colitis. In: Bockus, H. L. Gastroenterology. W. B. Saunders Co., Philadelphia, 1944, **2**, 549–614.
6. Bargen, J. A. Chronic Ulcerative Colitis. American Lecture Series, Publication No. 101. Charles C Thomas, Springfield, Ill., 1951.
7. Ricketts, W. E., and Palmer, W. L. Complications of chronic nonspecific ulcerative colitis. *Gastroenterology*, 1946, **7**, 55–56.
8. Editorial. Cirrhosis and colitis. *Brit. M. J.*, 1949, **1**, 189.
9. Bargen, J. A. Complications and sequelae of chronic ulcerative colitis. *Ann. Int. Med.*, 1929–30, **3**, 335–352.
10. Bargen, J. A. Functional and anatomic effects of colitis of long standing. *M. Times, New York*, 1936, **64**, 339–346 and 350.
11. Tumen, H. J., Monaghan, J. F., and Jobb, E. Hepatic cirrhosis as a complication of chronic ulcerative colitis. *Ann. Int. Med.*, 1947, **26**, 542–553.
12. Ross, J. R., and Swarts, J. M. Hepatic dysfunction and cirrhosis in chronic ulcerative colitis. *Gastroenterology*, 1948, **10**, 81–95.
13. Cabot cases 35101 and 35102. *New England J. Med.*, 1949, **240**, 384–390.
14. Warren, S., and Sommers, S. C. Pathogenesis of ulcerative colitis. *Am. J Path.*, 1949, **25**, 657–679.

15. Lansbury, J., and Bargen, J. A. The association of multiple hepatic abscesses and chronic ulcerative colitis. *M. Clin. North America*, 1933, **16**, 1427–1431.
16. Harrell, G. T., and McBryde, A. Cirrhosis of the liver in children. *Am. J. Dis. Child.*, 1940, **59**, 1301–1327.
17. Thompson, C. E., and Rice, M. L., Jr. Secondary amyloidosis in spinal cord injury. *Ann. Int. Med.*, 1949, **31**, 1057–1065.
18. Stewart, H. L., and Jones, B. F. Pathologic anatomy of chronic ulcerative cecitis: a spontaneous disease of the rat. *Arch. Path.*, 1941, **31**, 37–54.
19. Comfort, M. W., Bargen, J. A., and Morlock, C. G. The association of chronic ulcerative colitis (colitis gravis) with hepatic insufficiency: report of four cases. *M. Clin. North America*, 1938, **22**, 1089–1097.
20. McCannel, D. A. Perirectal infection and hepatic insufficiency complicating ulceration of the colon: report of case. *Proc. Staff Meet., Mayo Clin.*, 1939, **14**, 38–39.
21. Johnson, E. N., Jr. Hepatic insufficiency in chronic ulcerative colitis. *J. Bowman Gray School Med.*, 1947, **5**, 155–161.
22. Popper, H. Significance of agonal changes in the human liver. *Arch. Path.*, 1948, **46**, 132–144.
23. Gillman, T., and Chaikoff, I. L. Pathogenesis of experimental hepatic fibrosis and cirrhosis in the dog. *Arch. Path.*, 1949, **48**, 67–84.
24. Himsworth, H. P., and Glynn, L. E. Massive hepatic necrosis and diffuse hepatic fibrosis (acute yellow atrophy and portal cirrhosis): their production by means of diet. *Clin. Sc.*, 1944–45, **5**, 93–123.
25. Wahi, P. N. Diet and cirrhosis of the liver. *Arch. Path.*, 1949, **47**, 119–152.
26. Jankelson, I. R., and McClure, C. W. Chronic ulcerative colitis; deficiency states. *Rev. Gastroenterol.*, 1940, **7**, 506–510.
27. MacMahon, H. E., and Mallory, F. B. Streptococcus hepatitis. *Am. J. Path.*, 1931, **7**, 299–325.
28. MacMahon, H. E. Infectious cirrhosis. *Am. J. Path.*, 1931, **7**, 77–86.
29. MacMahon, H. E., and Mallory, F. B. Obstructive cirrhosis. *Am. J. Path.*, 1929, **5**, 645–657.
30. MacMahon, H. E., Lawrence, J. S., and Maddock, S. J. Experimental obstructive cirrhosis. *Am. J. Path.*, 1929, **5**, 631–643.
31. Ball, W. P., Baggenstoss, A. H., and Bargen, J. A. Pancreatic lesions associated with chronic ulcerative colitis. *Arch. Path.*, 1950, **50**, 347–358.
32. Hartley, G., Jr., and Lushbaugh, C. C. Experimental allergic focal necrosis of the liver. *Am. J. Path.*, 1942, **18**, 323–331.

DESCRIPTION OF PLATES

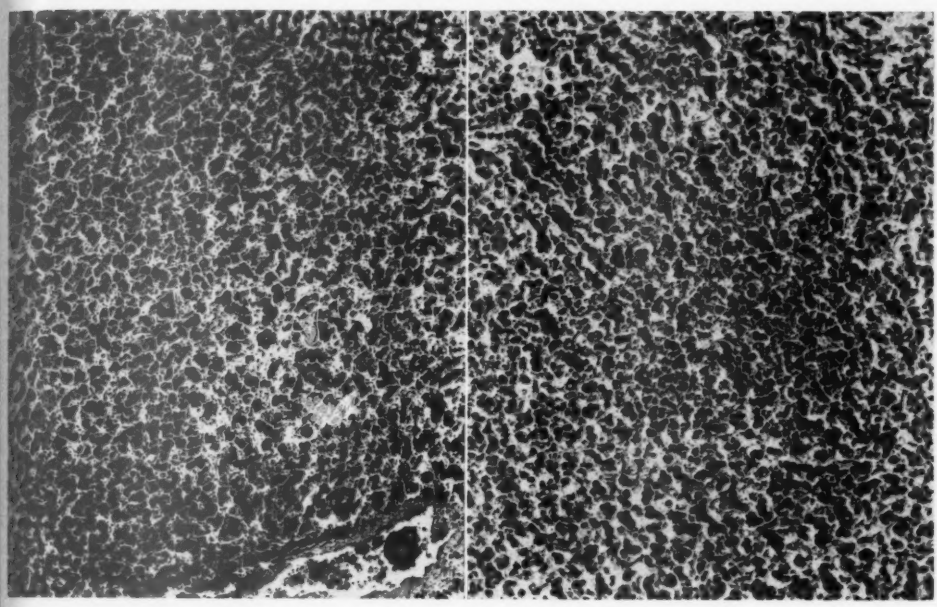
PLATE 34

FIG. 1. A–163. Advanced dissociation of liver cell cords, less marked in the periphery of the lobule. × 70.

FIG. 2. A–917. Severe diffuse dissociation of liver cell cords. × 70.

FIG. 3. A–242. Focal necrosis with associated moderate polymorphonuclear leukocytic infiltration. × 100.

FIG. 4. Armed Forces Institute of Pathology Accession 153167. Eccentric centrolobular necrosis with many bile pigmented macrophages and few polymorphonuclear leukocytes. The centrolobular vein is on the left. × 95.



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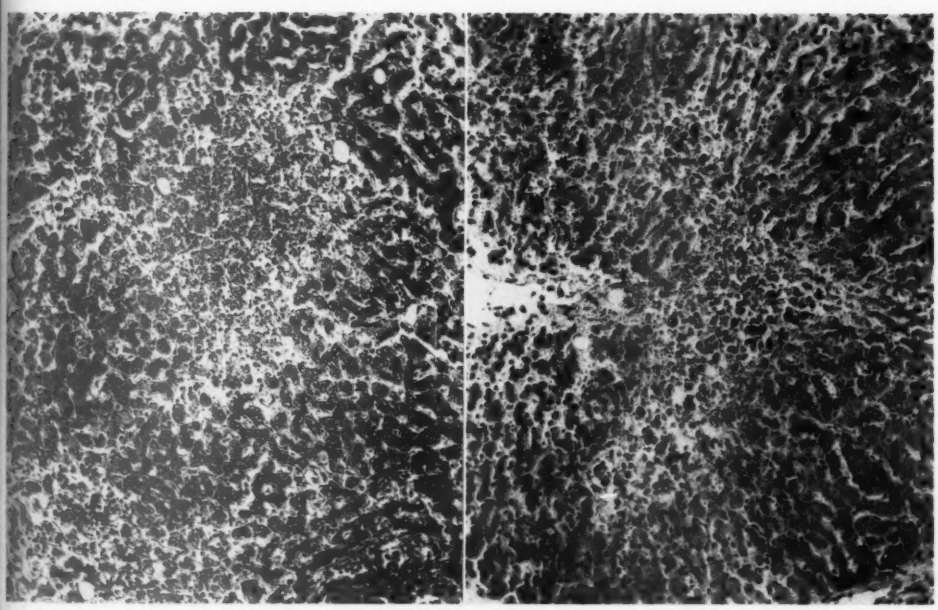


3 4

Kimmelstiel, Large, and Verner Liver Damage in Ulcerative Colitis

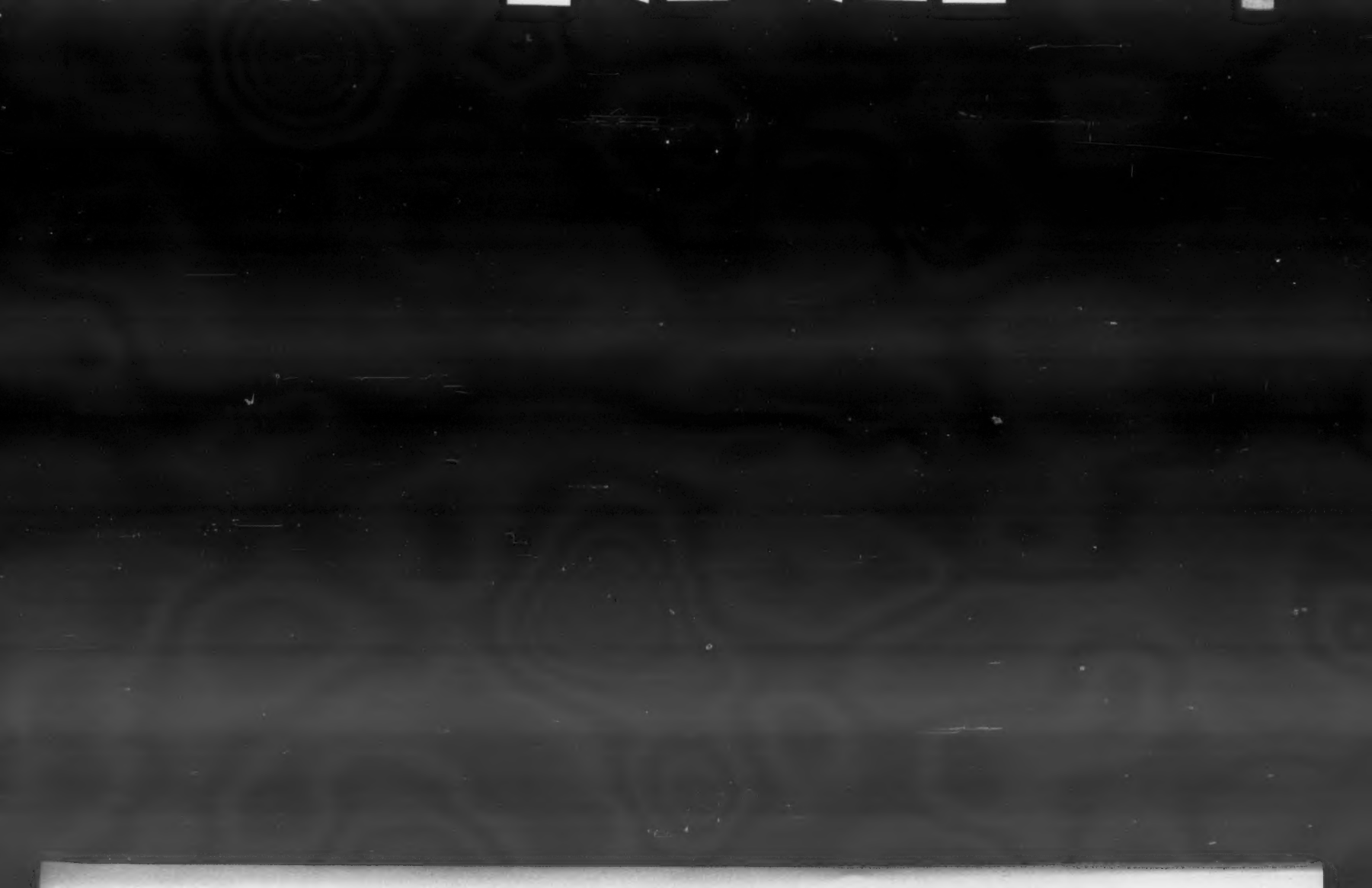
PLATE 35

FIG. 5. A–57. A large area of central necrosis has no inflammatory reaction about it. × 95.

FIG. 6. A–1089. Cirrhosis. × 75.

FIG. 7. A–15. Pseudolobulation. × 80.

FIG. 8. A–15. A focus of eccentric centrolobular fibrosis is adjacent to the central vein. × 115.



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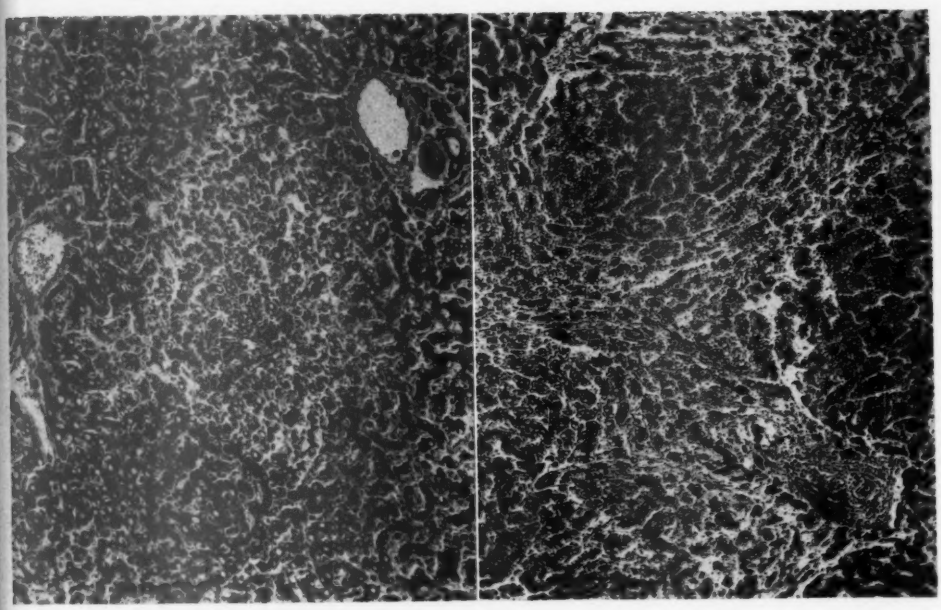

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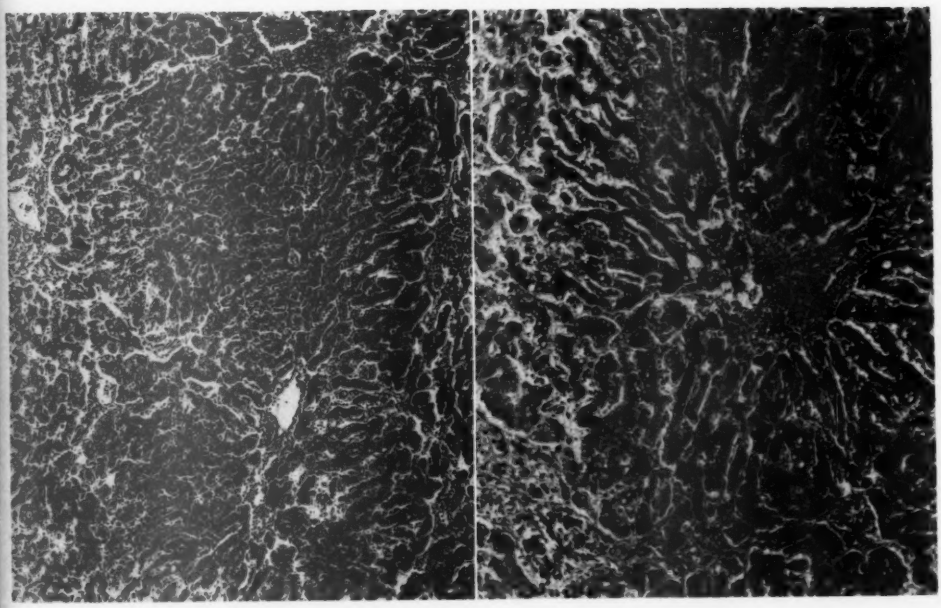


7 8

Kimmelstiel, Large, and Verner Liver Damage in Ulcerative Colitis

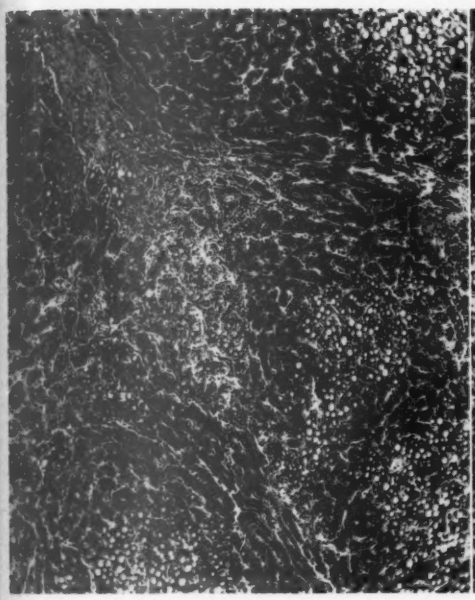
PLATE 36

FIG. 9. A–423. Conspicuous pseudolobulation with bile duct proliferation. × 60.

FIG. 10. A–423. Marked eccentric centrolobular fibrosis with fatty changes in liver cells. × 80.

FIG. 11. A.F.I.P. Acc. 190983. Several intracanalicular bile casts are present in a high-power field. × 305.

FIG. 12. A.F.I.P. Acc. 236758. A focus of polymorphonuclear leukocytic infiltration involves the wall of a centrolobular vein as well as the adjacent liver tissue. × 185.



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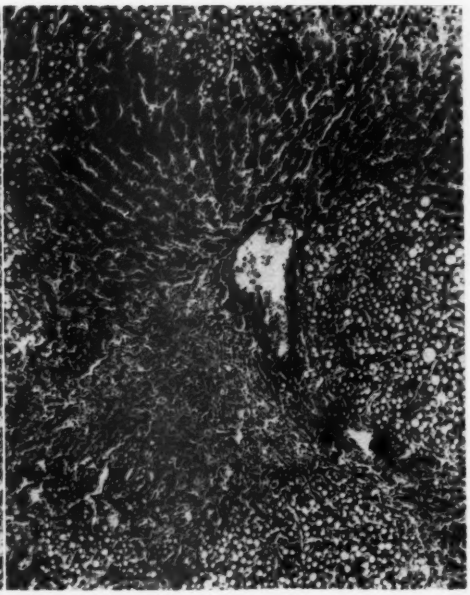

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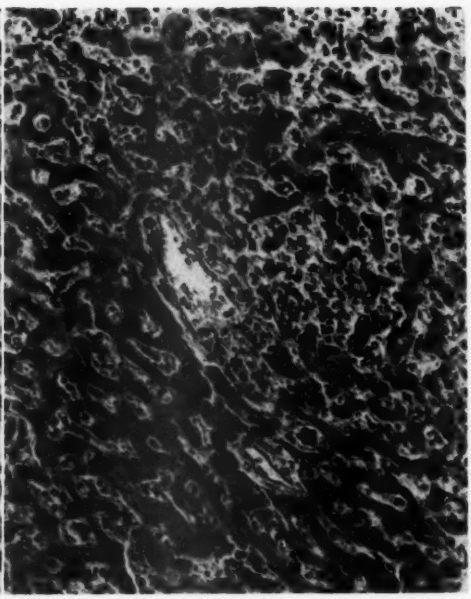


12

Kimmelstiel, Large, and Verner Liver Damage in Ulcerative Colitis

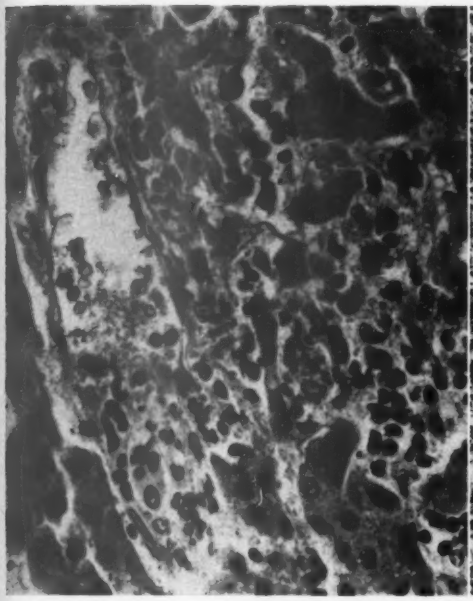
PLATE 37

FIG. 13. A.F.I.P. Acc. 236758. Leukocytic infiltration in and around the wall of the vein shown in Figure 12. × 390.

FIG. 14. A.F.I.P. Acc. 277125. Interlobular hepatitis with severe inflammatory infiltration in the periphery of hepatic lobules. × 40.

FIG. 15. A.F.I.P. Acc. 277125. High-power view of the upper right-hand corner of Figure 14, showing pleomorphic nature of leukocytic infiltration and predominance of polymorphonuclear leukocytes. × 195.

FIG. 16. A.F.I.P. Acc. 78852. Interlobular hepatitis with marked inflammatory infiltration on the left and an increase in the interlobular connective tissue on the right. Of note is the formation of pseudolobules in the center. × 75.



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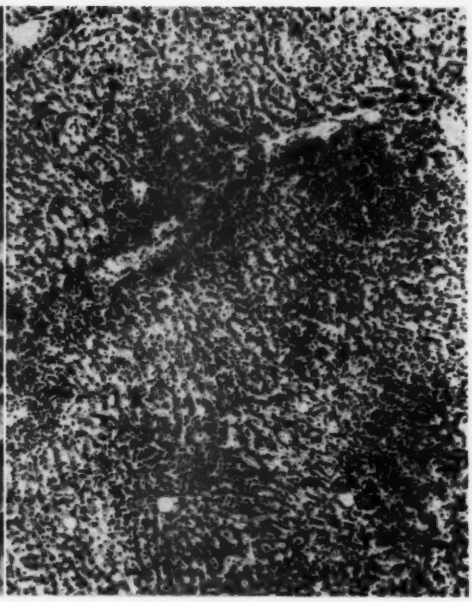

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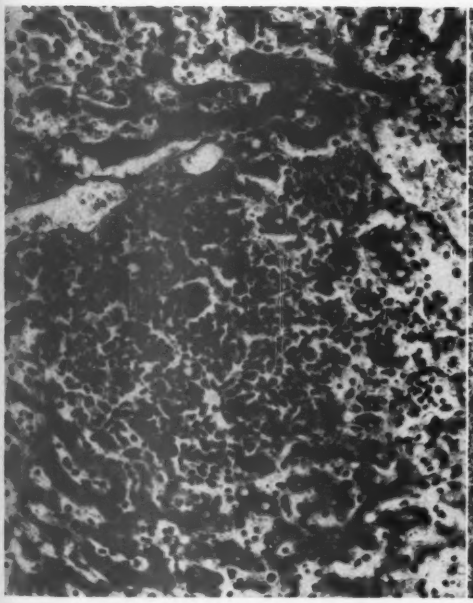

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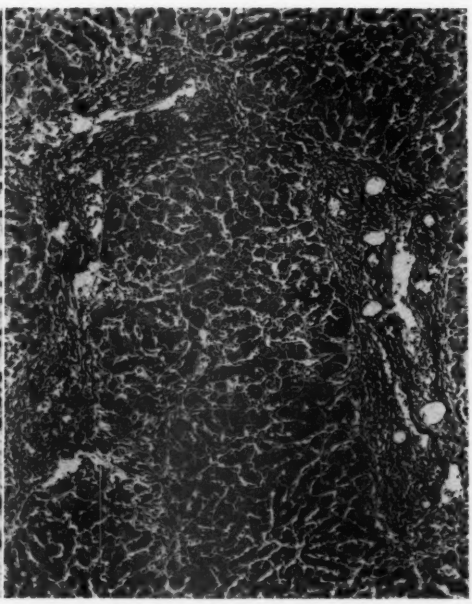

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Kimmelstiel, Large, and Verner

Liver Damage in Ulcerative Colitis

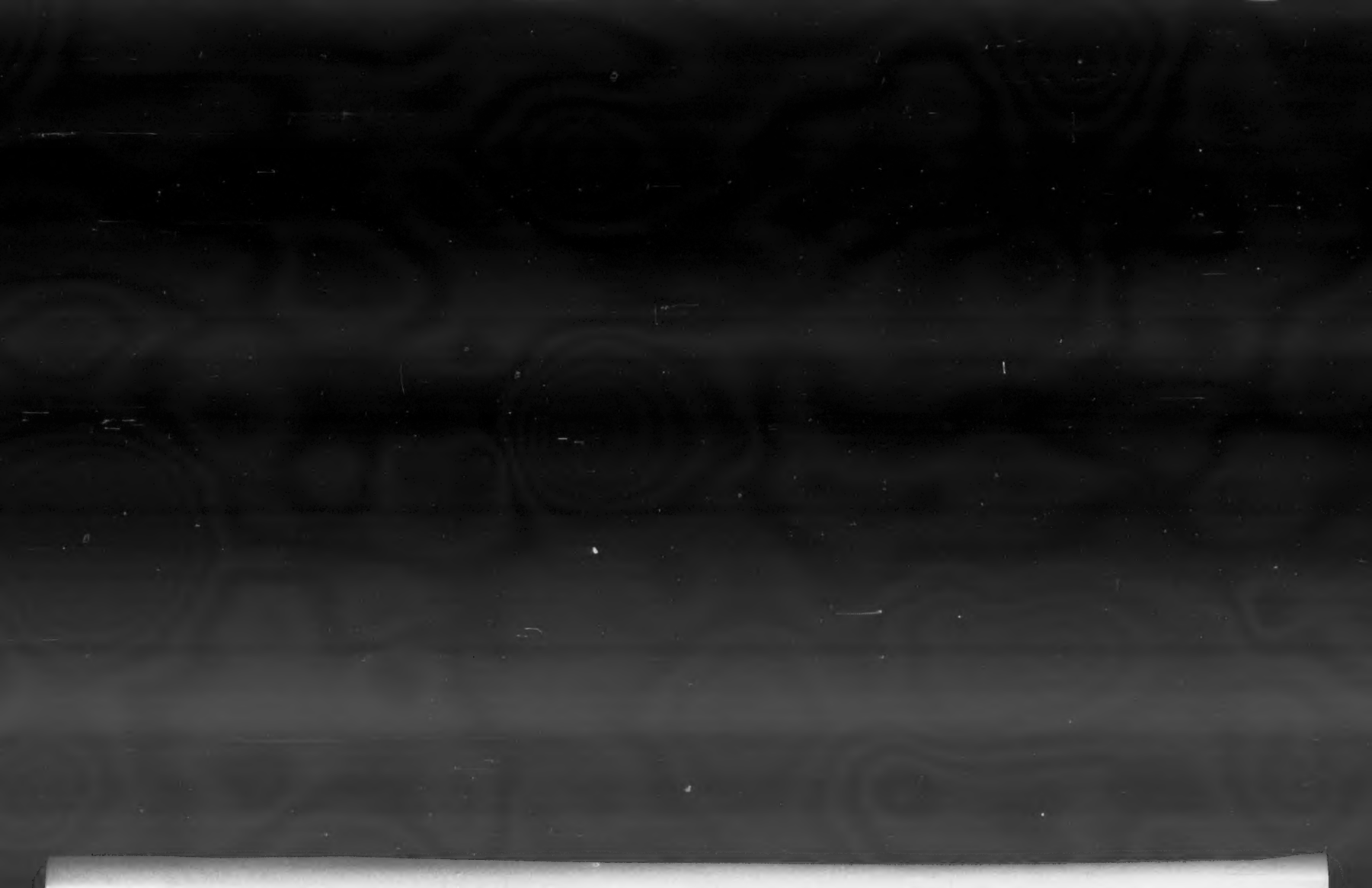
PLATE 38

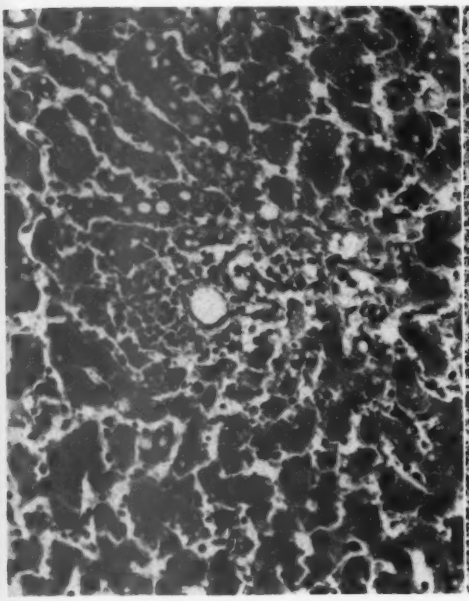
FIG. 17. A.F.I.P. Acc. 78852. Same case as seen in Figure 16, showing a solitary focus of lymphocytic infiltration about a centrolobular vein. × 195.

FIG. 18. A.F.I.P. Acc. 141078. Interlobular hepatitis, with marked widening of periportal spaces by a heavy pleomorphic inflammatory infiltration. There is some proliferation of bile ducts. × 70.

FIG. 19. A.F.I.P. Acc. 149528. Interlobular hepatitis. The portal triad is heavily infiltrated with a variety of small round cells. The infiltration extends into the periphery of adjacent lobules. × 75.

FIG. 20. A.F.I.P. Acc. 149528. Same case as seen in Figure 19, showing another area with more severe involvement of periphery of lobules including destruction of parenchymatous elements. × 80.





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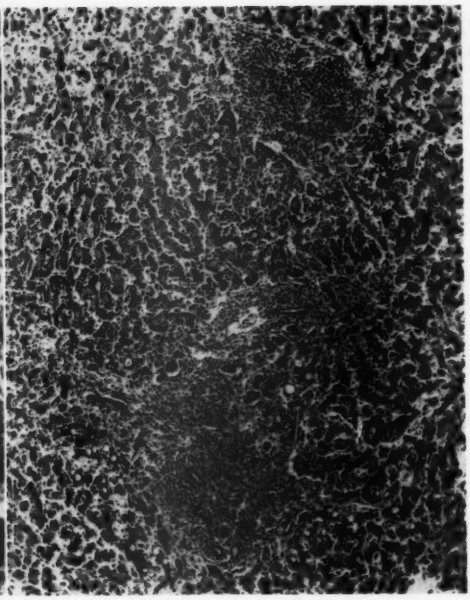

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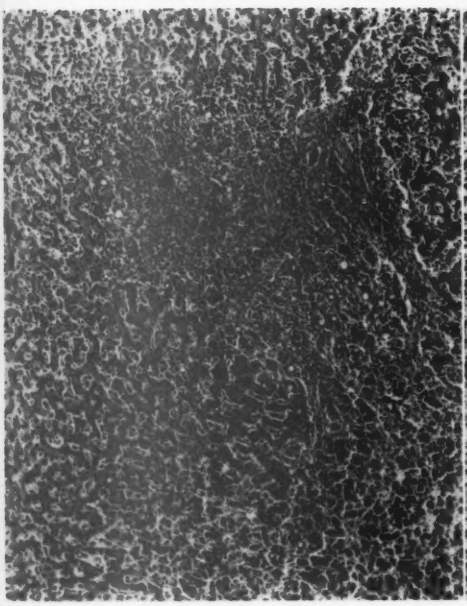

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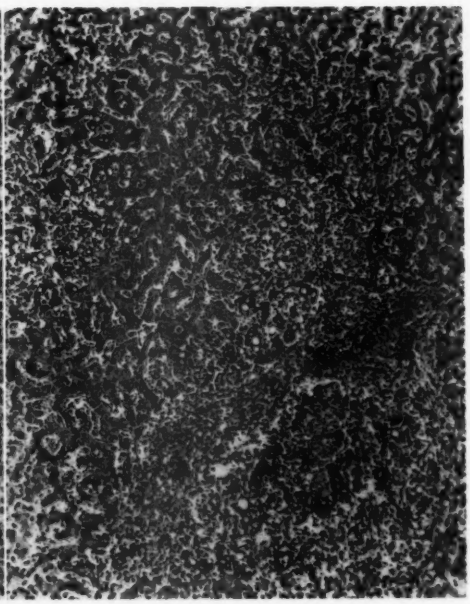

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Kimmelstiel, Large, and Verner

Liver Damage in Ulcerative Colitis

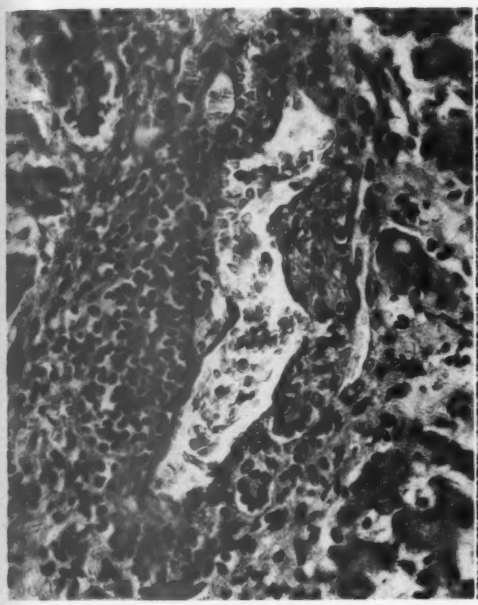
PLATE 39

FIG. 21. A.F.I.P. Acc. 149528. Same case as seen in Figures 19 and 20, with a focus of portal phlebitis with severe infiltration of wall of portal vein by lymphocytes and polymorphonuclear leukocytes. × 310.

FIG. 22. A.F.I.P. Acc. 269837. Focal interlobular hepatitis with severe infiltration of portal space with polymorphonuclear leukocytes and a few lymphocytes. × 110.

FIG. 23. A–860. Interlobular hepatitis, showing heavy round cell infiltration in periportal connective tissue, encroaching upon periphery of lobules. There is some fatty change in the liver cells. × 40.

FIG. 24. A–860. Same case as seen in Figure 23, with severe pleomorphic round cell infiltration in periportal space and in periphery of hepatic lobules. × 380.



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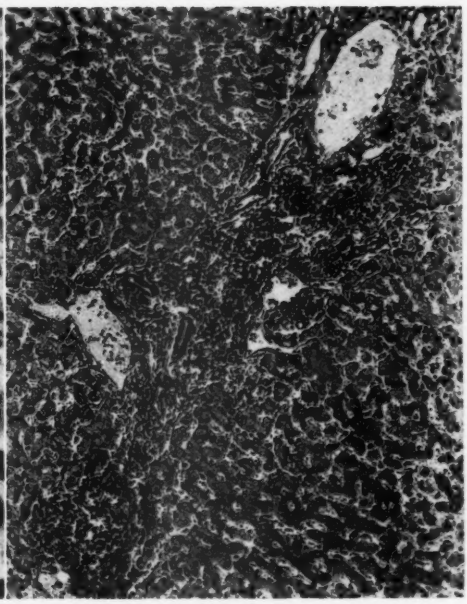

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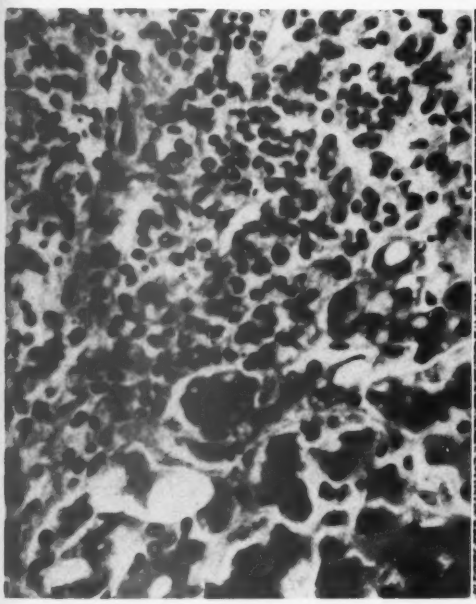

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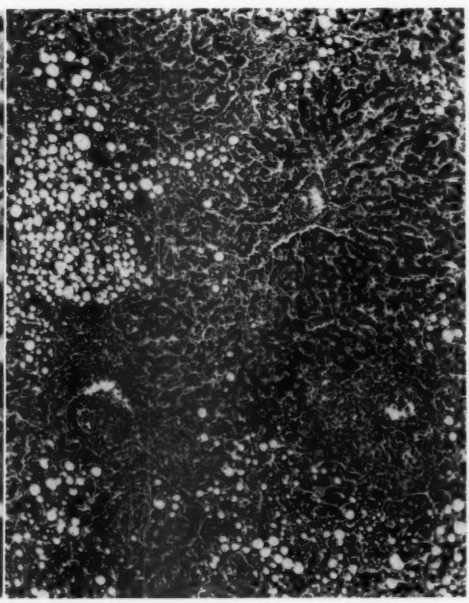

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Kimmelstiel, Large, and Verner

Liver Damage in Ulcerative Colitis

FURTHER STUDIES ON THE PATHOGENESIS OF URATE CALCULI IN THE URINARY TRACT OF WHITE RATS*

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In previous reports[1,2] it was observed that urate calculi were formed in rats after the development of coarsely nodular cirrhosis produced by the administration of carbon tetrachloride. In animals with finely nodular cirrhosis and with various non-fibrotic lesions of the liver, calculi were not seen.

The purpose of the following experiments was to study the pathogenesis of urate calculi in the urinary tract of white rats and to learn whether their formation and location is related to pathologic changes in this tract or to hepatic disease alone, or to a combination of changes in both systems.

Urate excretion in mammals (excluding man, anthropoid apes, and the Dalmatian dog) is presumed to be a result of disturbed uricolytic function of the liver, which, in turn, is dependent upon intact portal-venous circulation through the liver.[1-4] Uric acid calculi have been observed also in hepatic steatosis of extreme degree, which was produced by an experimental diet in dogs.[5] Other investigators have suggested that besides hepatic damage, factors like acidosis[6] or local injury in the urinary tract due to avitaminosis A[7] are related to the production of stones in animals excreting uric acid. The relationship between calculogenesis and secondary avitaminosis A in the presence of advanced cirrhosis had not been determined in our previous experiments. To investigate this further, the experiments were repeated on rats which were maintained on a vitamin A-deficient diet during the final period of treatment with carbon tetrachloride.

MATERIAL AND EXPERIMENTAL METHODS

Fifty male rats weighing 40 to 60 gm. received subcutaneous injections of CCl_4 (U.S.P. or B.D.H. analytic quality) twice weekly, 0.1 cc. per 100 gm. of body weight. The animals were at first maintained on a diet of wheat, skimmed milk, and tap water. Twice weekly, wheat germ and fresh vegetables were given. After fifty injections, milk and greens were withdrawn from this diet. The removal of milk was found to produce in adult animals the characteristic lesions of vitamin A deficiency. In one-half of the series, the administration of carbon tetrachloride was discontinued with the change of diet; the other half received twelve more injections.

* Received for publication, June 14, 1951.

At 4 to 8 weeks following withdrawal of milk and greens from the diet, 32 rats survived and were sacrificed. The bladders were opened, concretions collected for chemical examination, and the tissues preserved for histologic study. The kidneys were divided by cross sections through the smaller diameter into three fragments, so that the middle portion contained the papilla and the pelvis together with the proximal part of the ureter. After fixation in Carnoy's fluid, both kidneys of each animal were sectioned in "interrupted series" of eight to ten serial sections with intervals of about 50 μ. Sections were stained routinely with Mayer's hemalum and eosin, iron hematoxylin-van Gieson, and in selected cases with Sudan III following fixation in a 10 per cent solution of formaldehyde. In instances of suspected calcium deposits, von Kossa's reaction was applied. In rats in which urate calculi were found, sections of the kidneys were stained according to Schultz and Schmidt's method which is recommended as a stain for urates.[8]

Two series of animals were used as controls. One group of 24 rats (previously reported) received a complete diet and fifty injections of carbon tetrachloride. A second group of 10 animals was maintained on a complete diet without carbon tetrachloride injections.

Urinary pH was tested repeatedly from the 50th injection and the values ranged between 6 and 8. They never fell under 6, below which level Hammarsten and Lundgren[6] noted spontaneous formation of uric acid calculi in normal rats.

Results

BLADDER CALCULI

The calculi found in bladders were of two kinds: (1) Urate stones with traces of ammonium-magnesium phosphate, determined chemically. They were dark greenish brown, round or ovoid, with a smooth surface and measured 1 to 2 mm. in diameter. With strong pressure they broke into irregular fragments with fairly smooth surfaces. Ground disks, prepared and stained with hematoxylin or aniline dyes, showed concentric lamellae separated by narrow, homogeneous, basophilic rings. (2) Phosphate stones chemically analyzed as ammonium-magnesium phosphates. They were gray-yellow or white, irregular, and varied from less than 1 to 4 or 5 mm. in diameter (Fig. 1A). In one animal, concretions were found to contain traces of urates. In 6 of the 32 experimental rats the bladder contained urate calculi; in 5 others phosphate concretions were found; in the bladder of one animal both urate and phosphate concretions were present (Fig. 1B).

The findings in relation to the hepatic lesions are summarized in Table I.

HISTOLOGIC CHANGES IN BLADDERS AND KIDNEYS

Animals on Deficient Diet

Pathologic Changes in the Bladder. The bladders containing phosphate concretions from the 6 animals on the deficient diet showed varied lesions, from isolated ulcers to diffuse hemorrhagic necrotizing cystitis (Fig. 2). Similar severe changes were present in only one bladder with urate calculi. In the remaining animals, with urate concretions or without any stones, the bladder wall was thin except in the area of the trigone which appeared slightly roughened in a few rats. In the latter the mucosa

TABLE I
The Incidence of Calculi in Relation to the Type of Lesion in the Liver

Type of lesion in the liver	Number of animals	Calculi	
		Urate	Phosphate
I. Distortion of architecture; no fibrosis	11		1
II. Coarsely nodular hyperplasia; moderate increase of fibrous tissue	9	2	2
III. Finely nodular cirrhosis; moderate increase of fibrous tissue	8		2
IV. Coarsely nodular cirrhosis; marked fibrosis	4	4*	1*
Total number of animals	32	6	6

* One rat had both urate and phosphate calculi.

revealed isolated or confluent patches of squamous cell metaplasia with keratinization and occasional ulceration (Fig. 3). No relationship was determined between lesions of the bladder and the changes in the liver.

Pathologic Changes in the Kidneys. Calcium casts were present in the collecting tubules of 2 rats. In a few kidneys infiltrations of small lymphoid cells were seen between collecting tubules and adjacent to small blood vessels. In one animal there were also infiltrations of small lymphoid and a few plasma cells in the vicinity of several glomeruli. Similar infiltrations were present in the adipose tissue of the renal pelvis near the epithelial lining. The pelvis was slightly dilated in most kidneys. In rats with phosphate stones in the bladder the dilatation was seen as a bottle-like rounding of the fornix of the pelvis. The pelvic mucosa was made up of transitional epithelium, two to six layers in thickness. These alterations could not be correlated with any particular type of hepatic lesion.

In one animal partially calcified parasites were found within the pelvic epithelium (Fig. 4). The parasite was identified as *Capillaria*

(Trichosomoides) *crassicauda* by Professor G. Witenberg, helminthologist of our Medical School.

Significant changes, worthy of a detailed description, were seen in one kidney of one additional rat. In this animal phosphate calculi were found in the bladder and coarse nodular cirrhosis was present. The pelvis was markedly dilated, especially in the upper parts. It was lined by hyperplastic transitional epithelium, in some places forming eleven to fourteen layers of cells. Near the fornix on one side, neutrophilic leukocytes were scattered throughout the epithelial layers, and on the same side organized spherical formations were found lying free in the lumen or close to the epithelial surface (Fig. 5). In hematoxylin and eosin sections these formations showed unstained circular centers, measuring about 2 μ in diameter; they were surrounded by narrow, dark bluish, concentric rims alternating with eosinophilic layers of radially arranged "spikes." Such formations, when isolated, measured up to 30 μ in diameter (Fig. 6). In many instances they were fused in agglomerations of eight to ten centers. In some of them, the clear centers remained isolated from one another by dark basophilic rims, but in others, two or three clear, unstained centers were fused. The basophilic concentric rims remained unstained with von Kossa's method. The "spike-layers" and the clear centers were colored an intense green with the picric acid-methylene blue stain for sodium monourates.

Animals Maintained on Complete Diet and Treated with Carbon Tetrachloride

In the animals on a complete diet and treated with carbon tetrachloride no phosphate calculi were found and urate calculi were present only in animals having coarse nodular cirrhosis. The bladders showed no pathologic changes.

In the 24 pairs of kidneys examined, with the exception of one the findings were similar to those described above: there were calcium casts in some, and limited interstitial infiltrations with lymphoid cells in several other kidneys. In one animal a round calcified formation was found embedded in the epithelium of the lower segment of the pelvis. It measured about 35 μ in diameter and appeared structureless and basophilic when stained with hemalum and eosin or with von Kossa's procedure (Fig. 7). In the same kidney two, and in some sections three, spherical concretions were found in the pelvis, deeply stained with hemalum and densely impregnated with silver nitrate (Fig. 8). These formations lay in the fornix of the pelvis close to the

superficial layer of the epithelium; they were only slightly smaller than the one embedded in the epithelium and described above.

Rats Maintained on Complete Diet, Untreated with Carbon Tetrachloride (10 Rats)

Except for calcium casts in the collecting tubules of one kidney, no abnormal findings were noted in the untreated rats on a complete diet.

Discussion

The results of the present experiments suggest that urate calculi were formed in the lumen of the renal pelvis. This conclusion is based upon the evidence obtained from two animals. In one rat with coarsely nodular cirrhosis and hypovitaminosis A, the stratified spheres in the renal pelvis showed the same structure as those produced in test-tube experiments with uric acid[9] or with sulfa compounds and calcium oxalate.[10] Chemical analysis could not be made on these spheres and their composition was identified by staining properties. The picric acid-methylene blue stain recommended for staining of urates has been criticized as not being a true histochemical reaction,[11] but we have found it to be positive only in the kidneys of human newborn with uric acid deposits and negative for all other precipitations in rat kidneys in the present investigation. A second rat showed round concretions in the renal pelvis and urate calculi in the bladder. Hematoxylin and eosin sections revealed no specific structure of the pelvic concretions but ground disks of the bladder calculi had a lamellated appearance and staining properties similar to the stratified spheres mentioned above.

These findings demonstrate that urate calculi were formed in the lumen of the renal pelvis around minute centers of crystallization which correspond to the colloid micelles described by Hammarsten.[10]

The formation of urate calculi took place only in the presence of coarsely nodular cirrhosis and was independent of tissue lesions in the urinary tract. The findings do not support the opinion of Higgins[7] that the formation of an epithelial nidus during hypovitaminosis A may play a rôle in the growth of urate calculi. In rats with hepatic lesions maintained on a complete diet, there were no epithelial changes in the bladder and renal pelvis which might be attributed to secondary vitamin A deficiency. Moreover, in rats having lesions of hypovitaminosis A following the deficient diet, the incidence of urate calculi was not higher than that in animals on a complete diet. Phosphate calculi, on the other hand, were found only in the bladder and only in animals

with hypovitaminosis A. Nor can the other tissue lesions observed in the kidneys be regarded as a source of calculus formation. Interstitial renal inflammation has been reported as a not uncommon lesion in laboratory rats[12,13]; it was not recorded among changes resulting from experimental poisoning of rats with carbon tetrachloride.[14] In our experiments the calcium casts reached in rare instances to the papillary tip, but nowhere could we find the calcified lesion described by some authors[15,16] as the nidus for calculus formation.

Summary

Urate calculi have been observed in the urinary tract of rats having coarsely nodular cirrhosis of the liver, developed during the administration of carbon tetrachloride. The problem as to whether formation of the calculi is related to pathologic changes in the urinary tract in addition to hepatic diseases has been investigated.

The results of the present experiments show that urate calculi were formed in the lumen of the renal pelvis by precipitation of needle-like crystals radiating around minute amorphous centers, independent of tissue lesions in the urinary tract.

No relationship was found between the formation of urate calculi and hypovitaminosis A.

We wish to express our thanks to Professor E. Wertheimer and Dr. N. Herz of the Department of Pathological Physiology who performed the chemical examination of the calculi.

REFERENCES

1. Ungar, H. Experimental production of urate calculi in the urinary tract of white rats. *Brit. J. Exper. Path.*, 1945, **26**, 363–366.
2. Ungar, H. Transformation of the hepatic vasculature of rats following protracted experimental poisoning with carbon tetrachloride. Its possible relation to the formation of urate calculi in the urinary tract. *Am. J. Path.*, 1951, **27**, 871–883.
3. Fischler, F. J. Physiologie und Pathologie der Leber. Julius Springer, Berlin, 1925, ed. 2, 310 pp.
4. Hager, B. H., and Magath, T. B. The formation of vesical calculi. *J. A. M. A.*, 1928, **90**, 266–272.
5. Groen, J. An experimental syndrome of fatty liver, uric acid kidney stones, and acute pancreatic necrosis produced in dogs by exclusive feeding of bacon. *Science*, 1948, **107**, 425–426.
6. Hammarsten, G., and Lundgren, L. Über die experimentelle Erzeugung von Harnsteinen an der Ratte unter Azidose mit besonderer Berücksichtigung der Harnsäure- und Uratsteinbildung. *Lunds Universitets Årsskrift.*, N.F. 1940, Avd. 2, **36** (No. 16), 28 pp.
7. Higgins, C. C. Renal Lithiasis. Charles C Thomas, Springfield, Illinois, 1943, 140 pp.

8. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, p. 146.

9. Schade, H. Die physikalische Chemie in der inneren Medizin. Theodore Steinkopff, Dresden, 1923, ed. 3, 605 pp.

10. Hammarsten, G. The formation of the nucleus of stone in the urinary passages. *J. Path. & Bact.*, 1945, **57**, 375–378.

11. Lison, L. Histochimie Animale. Gauthier-Villars, Paris, 1936, 320 pp.

12. Jaffé, R. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Julius Springer, Berlin, 1931, 832 pp.

13. Humphrey, J. H. The pathogenesis of glomerulo-nephritis: a re-investigation of the auto-immunisation hypothesis. *J. Path. & Bact.*, 1948, **60**, 211–218.

14. Smyth, H. F., Smyth, H. F., Jr., and Carpenter, C. P. The chronic toxicity of carbon tetrachloride; animal exposures and field studies. *J. Indust. Hyg. & Toxicol.*, 1936, **18**, 277–298.

15. Lichtenstern, R. Mikrolithiasis. *Wien. klin. Wchnschr.*, 1926, **39**, 1156–1157.

16. Randall, A. Papillary pathology as a precursor of primary renal calculus. *J. Urol.*, 1940, **44**, 580–589.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 40

FIG. 1. Bladder calculi in rats maintained on deficient diet (about twice natural size). (A). Phosphate calculi. (B). Single large urate calculus (in lower half of the picture) and several small phosphate calculi from the same bladder.

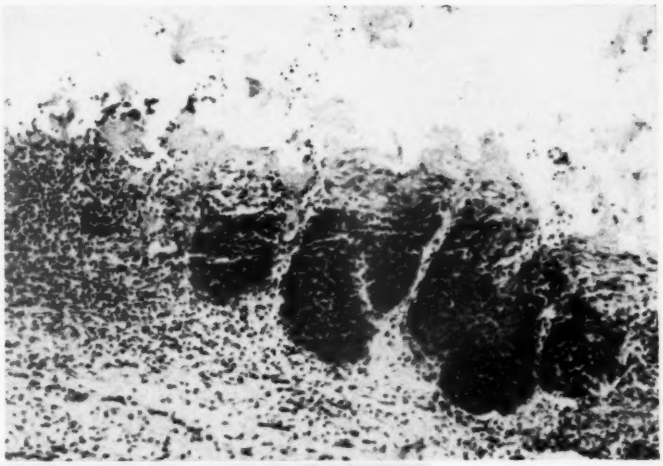
FIG. 2. Ulcerative cystitis with squamous cell metaplasia of mucosa. From rat on deficient diet, having phosphate calculi. Hematoxylin and eosin stain. × 120.

FIG. 3. Localized squamous cell metaplasia in the trigone of a bladder without calculi. Hematoxylin and eosin stain. × 320.

FIG. 4. Parasites (*Capillaria crassicauda*) in the epithelium of renal pelvis. Rat maintained on deficient diet, no calculi. Hematoxylin and eosin stain. × 400.

1A

1B



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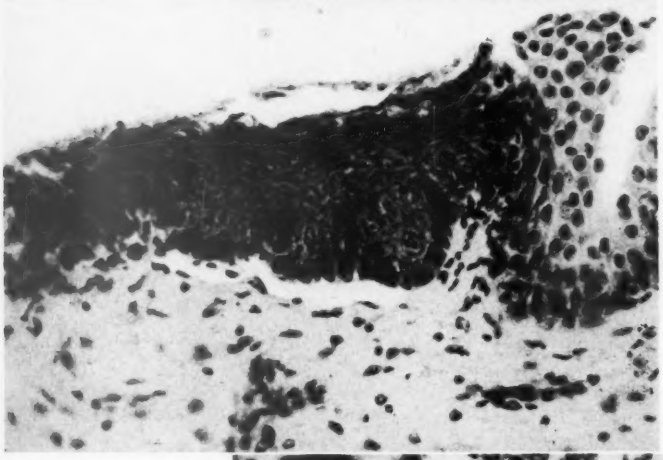

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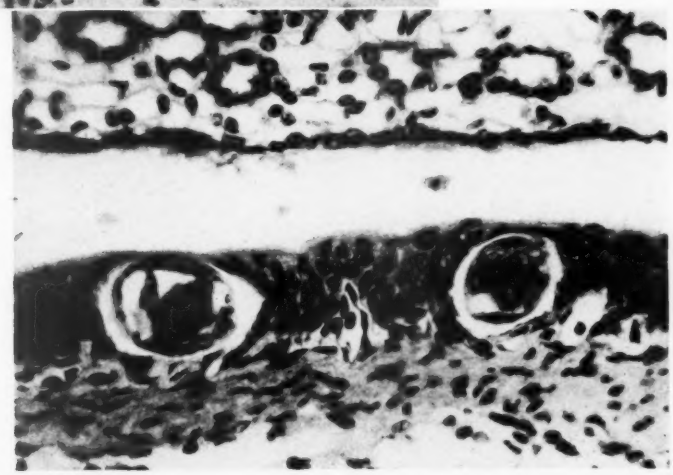

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PLATE 41

FIG. 5. Agglomeration of crystallization centers forming nuclei of urate calculi. From a rat with coarsely nodular cirrhosis, maintained on deficient diet. Hematoxylin and eosin stain. × 510.

FIG. 6. Crystallization center of urates. From the same rat as that from which Figure 5 was taken. Picric acid-methylene blue stain. × 1600.

FIG. 7. Focus of calcification in mucosa of renal pelvis. From a rat on the complete diet and having urate calculi in the bladder. Hematoxylin and eosin stain. × 450.

FIG. 8. Concretions in fornix of the renal pelvis. From the same rat as that from which Figure 7 was taken. Von Kossa's reaction-cresyl violet stain. × 300.

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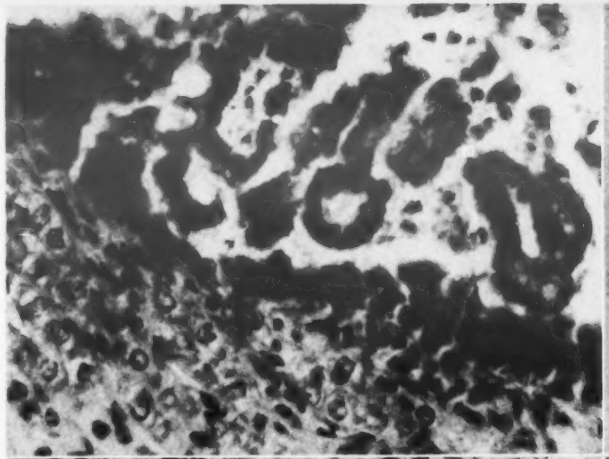

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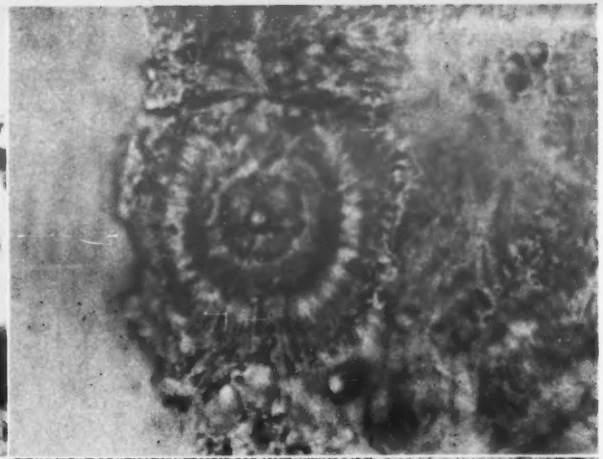

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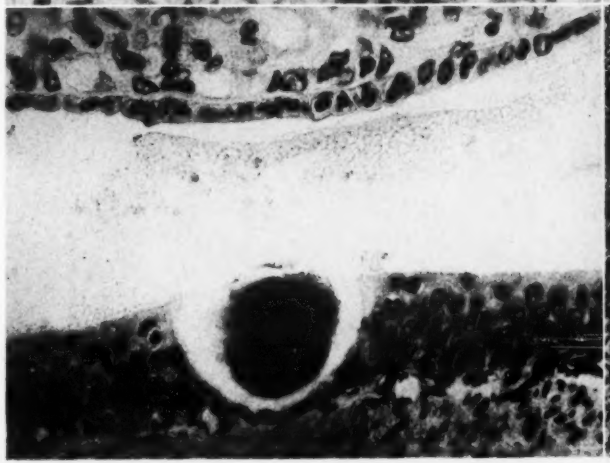

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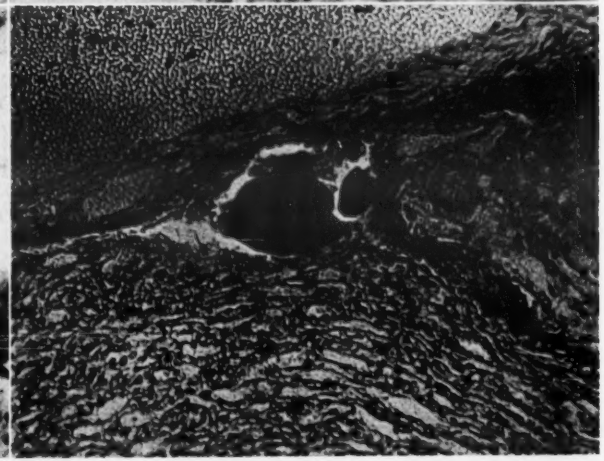

THE SPLEEN IN HYPERSPLENISM*

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The purpose of this paper is to describe a histopathologic change in the spleen in cases of hypersplenism. This change consists of a prominent marginal zone of medium and large lymphocytes at the periphery of the malpighian follicles. Awny[1] and Von Haam and Awny[2] described a similar feature as part of the diffuse and nodular reticuloendothelial hyperplasia in spleens from cases of hypersplenism. It is their opinion that the prominent marginal zone is composed of reticuloendothelial cells rather than lymphocytes.

A non-prominent marginal zone composed of medium and small lymphocytes is a normal component of the malpighian follicle in man.[3] This zone was termed the Knötchenrandzone by Weidenreich[4] in 1901, and the Follikelaussenzone by Strasser[5] in 1922. It is composed of a layer of pale, loosely arranged, larger lymphocytes peripheral to the mantle zone of small, dark lymphocytes. In certain animals, such as the woodchuck, the marginal zone is normally prominent,[3] but in this study of human spleens it was barely noticeable in most pathologic conditions or in the normal state.

The rôle of the spleen in the blood dyscrasias of Banti's syndrome, congenital hemolytic icterus, and idiopathic thrombocytopenic purpura has been appreciated for some time.[6] Recently, splenic neutropenia was described by Wiseman and Doan,[7] and splenic panhematopenia was reported by Doan and Wright.[8] However, with the possible exception of congenital hemolytic icterus, the histopathology of the spleen in cases of hypersplenism has been poorly understood and the findings have not served to distinguish hypersplenism from other conditions. Dameshek and Estren[6] reported that hypersplenism is a functional rather than an anatomical diagnosis. In this study it is shown that spleens of cases of hypersplenism contain prominent marginal zones of the malpighian follicles, a reliable histologic feature of moderately good specificity.

* The opinions and views set forth are those of the author and are not to be considered as reflecting the policies of the Navy Department.

The material on which this study was carried out was obtained from the Armed Forces Institute of Pathology, Washington, D.C., and the Pathology Department, Naval Medical School, Bethesda, Md.

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Material and Methods

The material for this study consisted of splenic tissue from 84 cases of hypersplenism. Most of these cases were obtained at the Armed Forces Institute of Pathology. There were 36 cases of congenital hemolytic icterus, 30 cases of idiopathic thrombocytopenic purpura, 9 cases of idiopathic splenic hematopenia, and 9 cases of Banti's syndrome. All of these cases had depression of one or more of the peripheral blood elements. When available for study, the bone marrow was hyperplastic. When the size of the spleen was known, it was greater than normal in all cases except in 7 cases of idiopathic thrombocytopenic purpura. In all cases in which the postoperative record of splenectomy was available there was some degree of improvement. In addition to anemia, splenomegaly, bone marrow hyperplasia, and improvement after splenectomy, cases of congenital hemolytic icterus were chosen with respect to spherocytosis, decreased resistance to hypotonic saline solution, icterus, and familial history. Cases of idiopathic thrombocytopenic purpura were chosen with special reference to hemorrhage, reduced platelets, prolonged bleeding time, non-retractile clot, positive tourniquet test, normal coagulation time, and absence of any cause of secondary thrombocytopenic purpura. There were 9 cases of hematopenia which had been diagnosed clinically as Banti's syndrome. Diagnostic features of Banti's syndrome, in addition to hematopenia, were splenomegaly, gastro-intestinal hemorrhage, cirrhosis of the liver, and absence of any other known cause for the hematopenia. The 9 cases of idiopathic hypersplenism were chosen on the basis of hematopenia, splenomegaly, bone marrow hyperplasia, improvement after splenectomy, and absence of any cause of secondary hematopenia.

The control series of 224 cases consisted of splenic tissue from necropsies and splenectomies. Most of these cases were selected at random; but some were selected by diagnosis to include rare diseases, and others were selected by age of the patient to include children. The latter two groups of cases were selected to study spleens in various diseases and possibly to discover diseases other than the hypersplenic syndrome which might show the prominent marginal zones of the malpighian follicles. Diagnoses in the control series of 224 cases included 68 cases of cancer, 65 of infectious disease, 29 of cardiovascular disease, and 13 spleens removed at splenectomy or necropsy following trauma. There were also 4 cases of secondary hemolytic icterus, 6 of secondary thrombocytopenic purpura, and other secondary hematopenias. All age groups were represented in the 224 control cases, 23

of which were between 9 months and 14 years of age. In addition, when the prominent marginal zones of the malpighian follicles were found in cases which showed no clinical evidences of hypersplenism, additional cases of these diagnoses were selected to determine the percentage which showed the feature. However, these additional cases were not included in the 224 cases of the control series.

Results

In the 36 cases of congenital hemolytic icterus, all showed the prominent marginal zones of the malpighian follicles (Figs. 1 and 3). The average weight of the spleen in 26 of the 36 cases in which actual weight was recorded was 937 gm., varying from 160 to 2280 gm. Congestion of the red pulp was prominent in all cases, extreme in most. Deposited hemosiderin was usually small in amount, but 6 spleens contained moderate amounts, and 4 contained large amounts. The malpighian follicles were uniformly small and widely separated. The germinal centers varied in size. A large number of sinuses in all cases were widely dilated and empty. Three spleens showed prominent reticulo-endothelial hyperplasia, but phagocytosis of red blood cells or peripheral blood elements of any type was not prominent.

In the 30 cases of idiopathic thrombocytopenic purpura, all showed prominent marginal zones of the malpighian follicles (Fig. 2). In one case, having less prominent marginal zones, several x-ray treatments to the spleen had been given prior to splenectomy. The average weight of the spleen in 20 of the 30 cases in which the actual weight was recorded was 197 gm., varying from 23 to 375 gm. In all but 2 of the 30 cases of idiopathic thrombocytopenic purpura, there were germinal centers in the malpighian follicles. The majority of these germinal centers were large. Other histopathologic features were not consistent. There were varying degrees of reticulo-endothelial hyperplasia and congestion of the red pulp. In no case were megakaryocytes noted in the sinuses.

In the remaining 18 cases of hypersplenism, which included 9 of Banti's syndrome and 9 of idiopathic hypersplenism, the prominent marginal zones of the malpighian follicles were present (Fig. 4) in all but one. In this one case of Banti's syndrome, the patient had received x-ray treatment to the spleen prior to splenectomy, with atrophy of the malpighian follicles. The remaining 8 of the 9 cases of Banti's syndrome showed prominent splenic trabeculae, fibrosis of the pulp cords, small malpighian follicles, and evidence of old hemorrhage. In the spleens of the 9 cases of idiopathic hypersplenism, there was congestion of the red pulp with dilatation of the sinuses. One case showed mod-

erate fibrosis of the pulp cords and increased hemosiderin deposition. Reticulo-endothelial hyperplasia was present to a varying degree in the spleens of all cases of idiopathic hypersplenism but increased phagocytosis was not noted.

Two hundred and twenty-four control cases were studied to determine whether the prominent marginal zones of the malpighian follicles were present in diseases other than hypersplenism. An attempt was made to include diverse pathologic conditions in this control series.

Thirteen, or 5.8 per cent, of the 224 control cases showed the prominent marginal zones of the malpighian follicles (Figs. 5 and 6). These were quite irregular and poorly defined in most cases, but they were definite enough to be confused with the marginal zones of cases of hypersplenism. Of these 13 cases, there were 8 cases of hemorrhage into the spleen, including 7 ruptured spleens and one case of hemophilia. A total of 18 cases of ruptured spleen were collected to determine the percentage which showed the prominent marginal zones. Of these, 9 showed the prominent marginal zones of the malpighian follicles and 9 did not. The only clinical difference between those cases with and without prominent marginal zones was the length of time between the injury and splenectomy or death. The prominent marginal zones were present in cases in which the interval was 7 hours to 17 days, but the prominent marginal zones were absent in cases in which the interval was less than 5 hours with but one exception. In this case there were pains suggestive of splenic rupture 2 days prior to splenectomy. Twelve cases of amyloidosis were examined and 3 showed the prominent marginal zones of the malpighian follicles. These 3 cases were of the early focal type of amyloidosis. Of the 9 spleens with amyloidosis which did not show the prominent marginal zones, there were 4 of the early focal type which had a suggestion of prominent marginal zones. The remaining 2 of the 13 control cases showing the prominent marginal zones of the malpighian follicles were examples of acute laryngitis with asphyxia in a 3-year-old child, and status lymphaticus with cessation of respiration after anesthesia in a 6-year-old child. Thirty-one other cases of cardiac and pulmonary disease with right heart strain did not show the prominent marginal zones, but this feature was present in one case of aneurysm of the pulmonary artery with severe pulmonary arteriosclerosis and right heart failure, in one of hypertensive cardiovascular disease with emphysema, and in one of emphysema with congestive heart failure. There was a suggestion of prominent marginal zones of the malpighian follicles in 3 cases of rheumatic heart disease, in one of status asthmaticus, in 3 of hypertensive

cardiovascular disease, in one of cor pulmonale with emphysema, and in one of heart failure with emphysema.

Discussion

The histopathology of the spleen in cases of hypersplenism has been generally regarded as non-specific. The microscopic picture of congenital hemolytic icterus, although fairly constant in this type of hypersplenism, is the same as that of the secondary hemolytic anemias.[9] Dick[10] described the splenic lesions of hemolytic anemia in 1925. He found extreme congestion, dilated sinuses, small malpighian follicles, absence of phagocytosis, and small amounts of hemosiderin pigment. Diverse histologic descriptions have been reported in spleens from cases of idiopathic thrombocytopenic purpura. Gordon[11] found a uniform microscopic picture in which the malpighian follicles were not changed. He described phagocytic giant cells and proliferation of the sinus endothelium. McLean, Kreidel, and Caffey[12] examined the spleens from 7 splenectomies and 3 necropsies of cases of idiopathic thrombocytopenic purpura. There was moderate congestion of the sinuses in 2 cases and hyperplasia of the malpighian follicles in one. The number of platelets in the splenic sinuses varied from complete absence to large numbers of platelet masses. In a series of 57 splenectomies for idiopathic thrombocytopenic purpura, Pemberton[13] found on microscopic examination no constant or distinctive pathologic changes except chronic congestion and chronic splenitis. On histologic examination of 11 spleens in cases of idiopathic thrombocytopenic purpura, Nickerson and Sunderland[14] found enlarged and active germinal centers in all but 2. The germinal centers in 2 cases showed areas of necrosis of lymphocytes, occasional infiltration of polymorphonuclear leukocytes, and clumps of fibrin. In all cases but 2 there was an increase of neutrophilic or eosinophilic polymorphonuclear leukocytes in the sinuses. Megakaryocytes were present in the sinuses in almost all cases, although they were rare in most instances. Wiseman, Doan, and Wilson[15] found no noteworthy distinguishing features on microscopic examination of spleens of cases of idiopathic thrombocytopenic purpura. Austoni[16] reported an increased number and size of the malpighian follicles with the presence of large germinal centers in idiopathic thrombocytopenic purpura. The histologic pictures of splenic neutropenia and splenic panhematopenia were described by Wiseman and Doan[7] and Doan and Wright[8] from direct supravital studies of the freshly removed spleen. They found a marked increase in the number and phagocytic activity of macrophages which were acting to destroy

the diminished peripheral blood elements. Heinle and Holden,[17] however, did not find excessive phagocytosis of the myeloid elements in a study of 7 spleens from cases of splenic panhematopenia.

The exceptional prominence of the marginal zones of the malpighian follicles as seen in all of the cases of hypersplenism studied except one, and the relative absence of this characteristic in a series of cases without hypersplenism, would suggest a possible use of this histopathologic feature in the diagnosis or exclusion of hypersplenism. The only case of hypersplenism which did not show the prominent marginal zones in the spleen had received irradiation to the splenic area and the malpighian follicles were atrophic. Since the prominent marginal zones cannot be present when the malpighian follicles have atrophied, the latter case is excluded. In the 83 remaining cases of hypersplenism, the prominent marginal zones were conspicuous. The most striking characteristic was the well delineated nature of the pale rim which surrounded the malpighian follicle and separated it from the red pulp (Figs. 1 to 4). In contrast, even the most prominent marginal zones of the cases without clinical evidences of hypersplenism were not of the well delineated character seen in the spleens of cases of hypersplenism (Figs. 5 and 6). In most of the control cases which showed the prominent marginal zones, the lymphocytes were of only moderate size, and the marginal zone merged gradually with the red pulp rather than being separated from it.

All lymphocytes of the malpighian follicle, including those of the mantle zones, were rather large in certain diseases such as severe infections or terminal cases of malignancy. Also, in many different conditions such as infection, heart and lung disease, and disease in children, there was prominence of part of the marginal zone due to the presence of medium lymphocytes in that segment. However, the marginal zones of the malpighian follicles of these spleens were clearly discernible from the well delineated, circumferential, prominent marginal zones in spleens of cases of hypersplenism.

The actual presence, or a suggestion of the presence, of prominent marginal zones in spleens from patients who did not clinically show evidences of the hypersplenic syndrome suggests that various degrees of splenic hyperfunction may be part of many diseases.

Conclusion

Eighty-three spleens from patients exhibiting the hypersplenic syndrome and 224 spleens from cases other than hypersplenism were examined microscopically. All of the spleens of cases of hypersplenism showed prominent marginal zones of the malpighian follicles, but only

13, or 5.8 per cent, of the control cases showed the prominent marginal zones.

The prominent marginal zones of the malpighian follicles of the spleen may be of value as a diagnostic feature of hypersplenism.

REFERENCES

1. Awny, A. J. The pathology of hypersplenic syndromes. *J. Roy. Egyptian M. A.*, 1947, **30**, 513–524.
2. Von Haam, E., and Awny, A. J. The pathology of hypersplenism. *Am. J. Clin. Path.*, 1948, **18**, 313–322.
3. Klemperer, P. The Spleen. In: Downey, H. Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, **3**, 1591–1754.
4. Weidenreich, F. Das Gefässsystem der menschlichen Milz. *Arch. f. mikr. Anat.*, 1901, **58**, 247–376.
5. Strasser, U. Zur Hämosiderosefrage nebst Beiträgen zur Ortho- und Pathohistologie der Milz. *Beitr. z. path. Anat. u. z. allg. Path.*, 1922, **70**, 248–264.
6. Dameshek, W., and Estren, S. Hypersplenism. *M. Clin. North America*, 1950, **34**, 1271–1289.
7. Wiseman, B. K., and Doan, C. A. Primary splenic neutropenia; a newly recognized syndrome, closely related to congenital hemolytic icterus and essential thrombocytopenic purpura. *Ann. Int. Med.*, 1942, **16**, 1097–1117.
8. Doan, C. A., and Wright, C. S. Primary congenital and secondary acquired splenic panhematopenia. *Blood*, 1946, **1**, 10–26.
9. Thompson, W. P. The splenic lesion in hemolytic jaundice. *Bull. Johns Hopkins Hosp.*, 1932, **51**, 365–370.
10. Dick, W. Die histologischen Befunde bei einem Fall von hämolytischem Ikterus. *Med. Klin.*, 1925, **21**, 1309–1311.
11. Gordon, A. K. The rôle of the spleen in the causation of haemorrhage. *Tr. Roy. Soc. Trop. Med. & Hyg.*, 1928–29, **22**, 309–334.
12. McLean, S., Kreidel, K., and Caffey, J. Hemorrhagic thrombocytopenia in childhood. A clinical study of twenty-one cases. *J. A. M. A.*, 1932, **98**, 387–393.
13. Pemberton, J. deJ. The diagnosis and treatment of purpura hemorrhagica. *Am. J. Surg.*, 1934, **24**, 793–806.
14. Nickerson, D. A., and Sunderland, D. A. The histopathology of idiopathic thrombocytopenic purpura hemorrhagica. *Am. J. Path.*, 1937, **13**, 463–489.
15. Wiseman, B. K., Doan, C. A., and Wilson, S. J. The present status of thrombocytopenic purpura with special reference to diagnosis and treatment. *J. A. M. A.*, 1940, **115**, 8–13.
16. Austoni, M. Sindromi werlhoffiane e iperplasia dei follicoli splenici. *Clin. nuova*, 1948, **6**, 180–190.
17. Heinle, R. W., and Holden, W. D. Primary splenic panhematopenia. *Surg., Gynec. & Obst.*, 1949, **89**, 79–91.

[*Illustrations follow*]

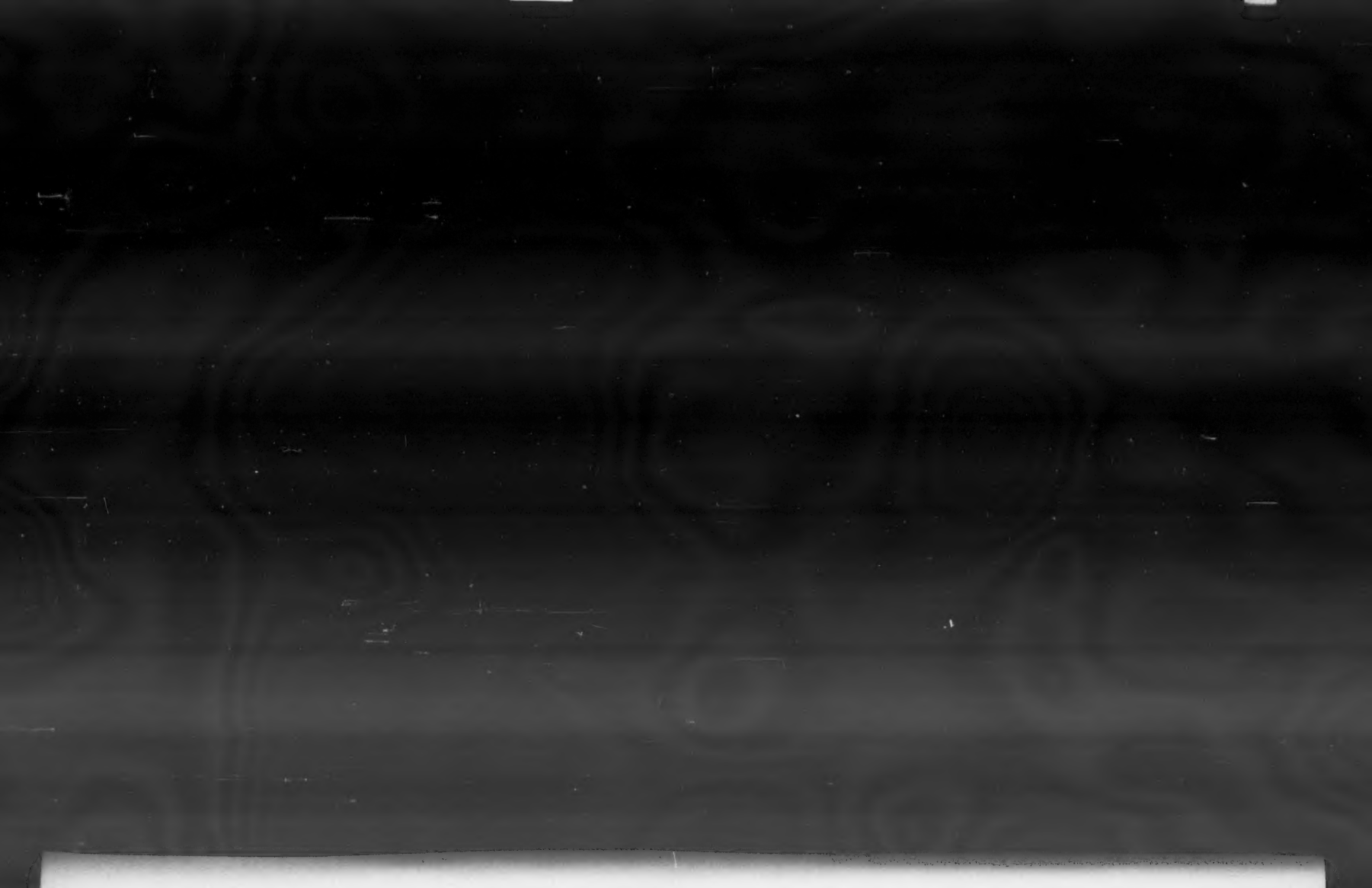
DESCRIPTION OF PLATES

PLATE 42

FIG. 1. Malpighian follicle in the spleen of a case of congenital hemolytic icterus showing the prominent marginal zone. × 40.

FIG. 2. Malpighian follicle in the spleen of a case of idiopathic thrombocytopenic purpura showing the prominent marginal zone. × 40.

FIG. 3. Marginal zone in the malpighian follicle of a case of congenital hemolytic icterus. × 300.



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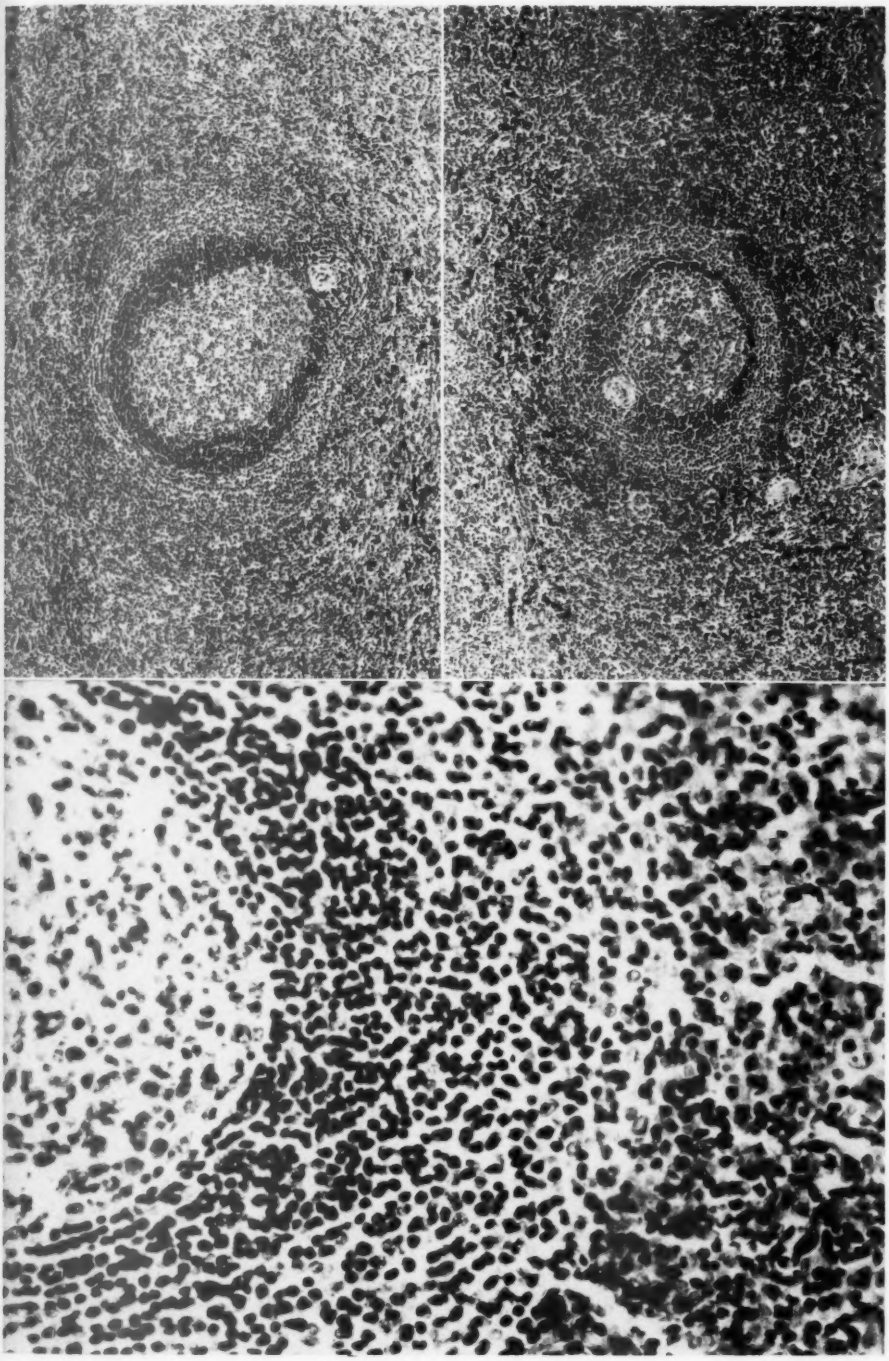


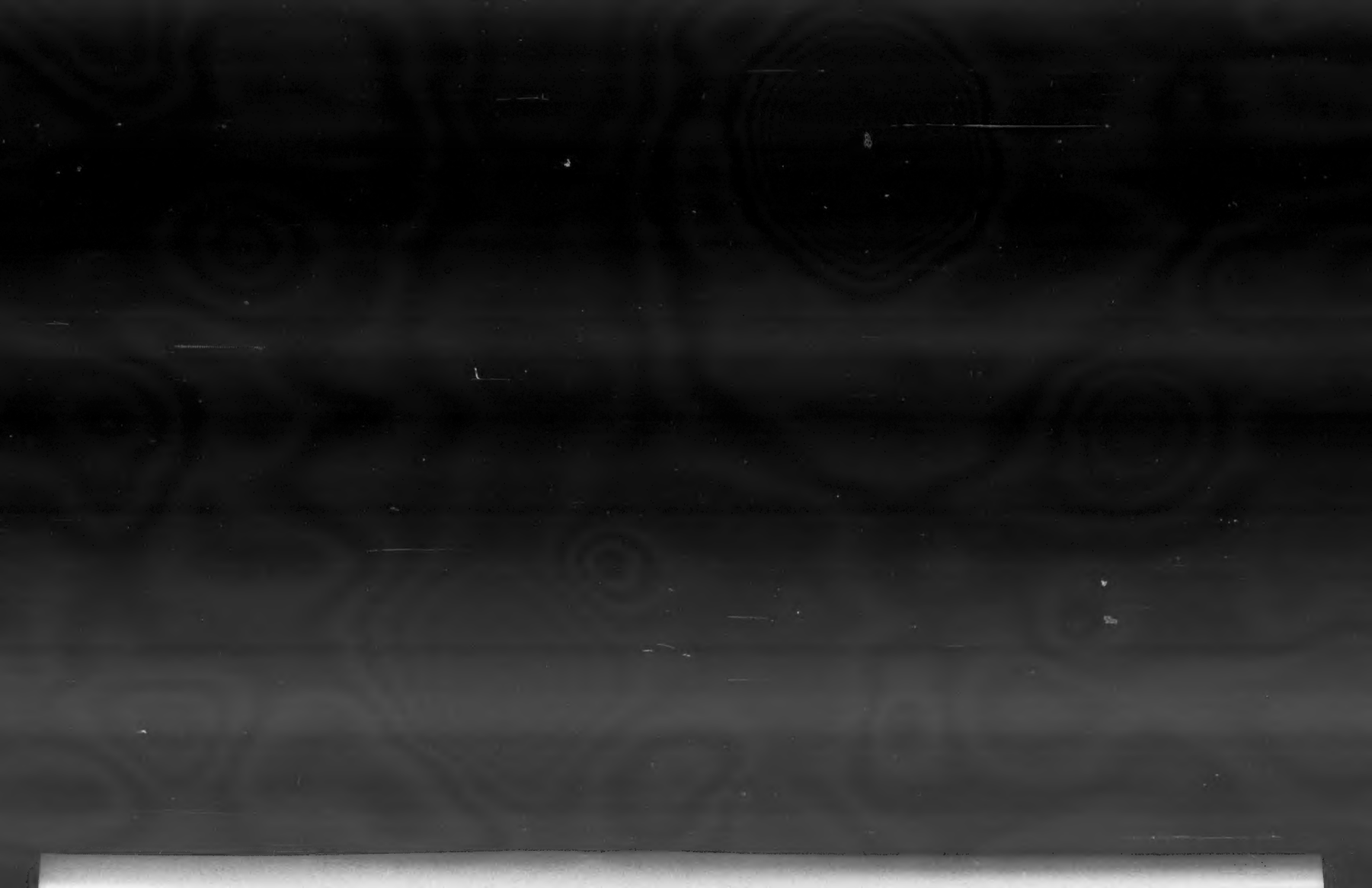
Leffler Hypersplenism

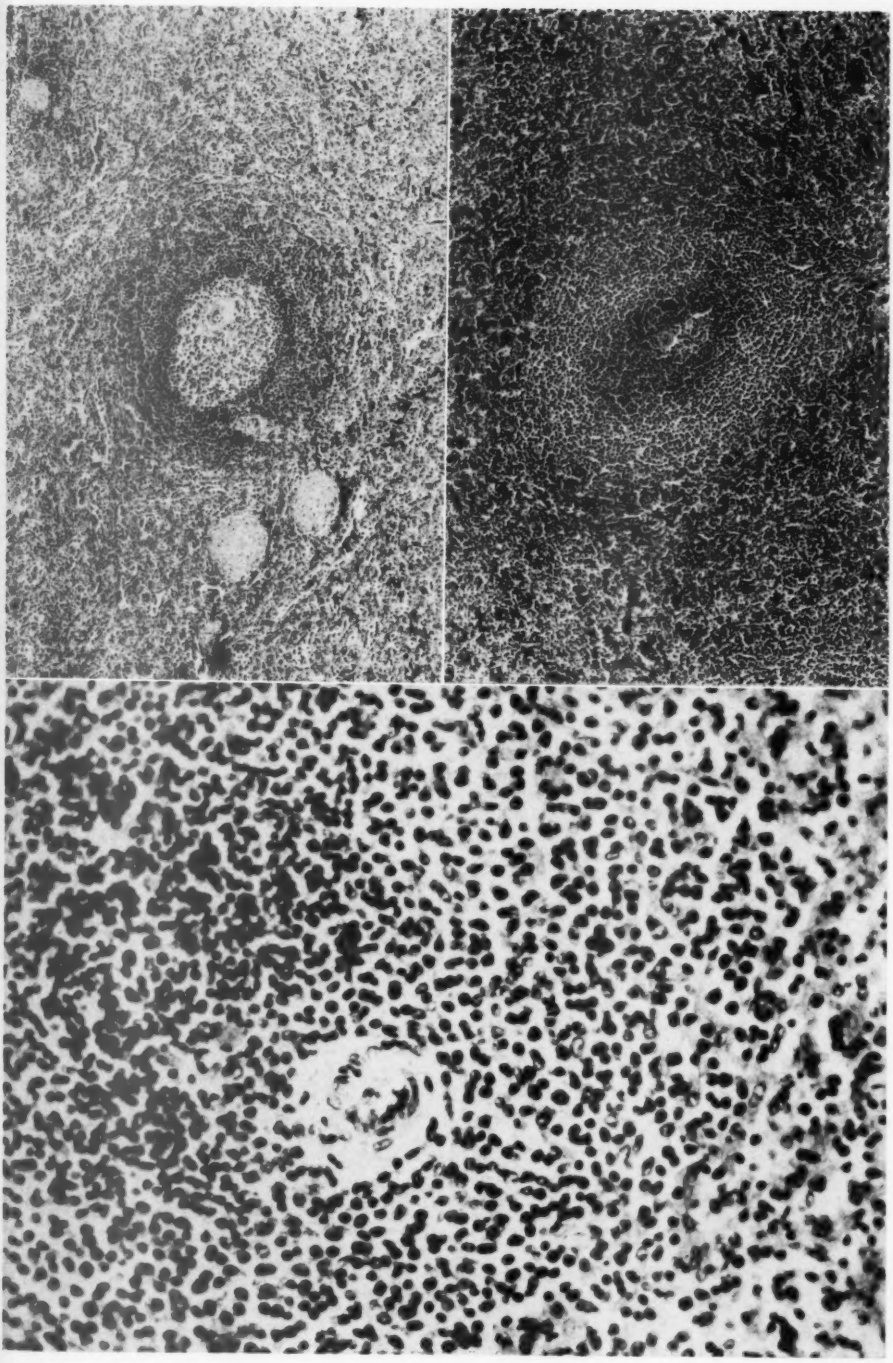
PLATE 43

FIG. 4. Malpighian follicle in the spleen of a case of idiopathic hypersplenism showing the prominent marginal zone. × 40.

FIG. 5. Malpighian follicle in the spleen of a case of aneurysm of the pulmonary artery with right heart failure, showing the prominent marginal zone. This represents the greatest degree of prominence in the cases without clinical evidence of hypersplenism. × 40.

FIG. 6. Marginal zone in the malpighian follicle of a case of aneurysm of the pulmonary artery with right heart failure. × 300.





Leffler Hypersplenism

LIPID DEPOSITION IN AORTAS IN YOUNGER AGE GROUPS FOLLOWING CORTISONE AND ADRENOCORTICOTROPHIC HORMONE*

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This investigation was stimulated by the finding of an excessively large amount of intimal lipid in the aorta of an 8-year-old child who had been treated with cortisone. In view of the effect of the adrenal cortex on cholesterol and other serum lipids[1-5] and the assumed relationship between cholesterol metabolism and atherosclerosis,[6] it was decided to investigate the changes in the aortas of patients who had received cortisone and/or adrenocorticotrophic hormone.

MATERIALS AND METHODS

This study was restricted to patients under 21 years of age because lipid deposits of more than minimal degree are found in the aortic intima almost universally after the second decade. Thirty-four cases which had been treated with cortisone or ACTH were collected at the Memorial Hospital for Cancer and Allied Diseases in New York and at the University of Virginia Hospital. Most of these patients had died of leukemia. Forty-three control cases were selected from patients who had received no cortisone or ACTH. These patients had died of diverse causes and the control group included 12 cases of leukemia. The samples of aortas were selected at random.

Hematoxylin and eosin, Weigert's elastic tissue, Sudan IV, Schultz' method for sterols,[7] and periodic acid-Schiff's reagent for 1,2 glycols, including polysaccharides,[8] were the stains used. The material was arranged in four groups, each including a 5-year age period.

RESULTS

Intimal lipid deposition of moderate or marked degree was found in the first age group in 9 of the 15 treated cases (Text-fig. 1). There was no treated case without some degree of lipid deposition. Eleven of the 15 controls had no lipid and in no case was there more than a minimal amount.

Comparable differences were observed between the treated and control cases in the 6 to 10 year age group.

After the age of 11 years, intimal lipid was found so frequently in

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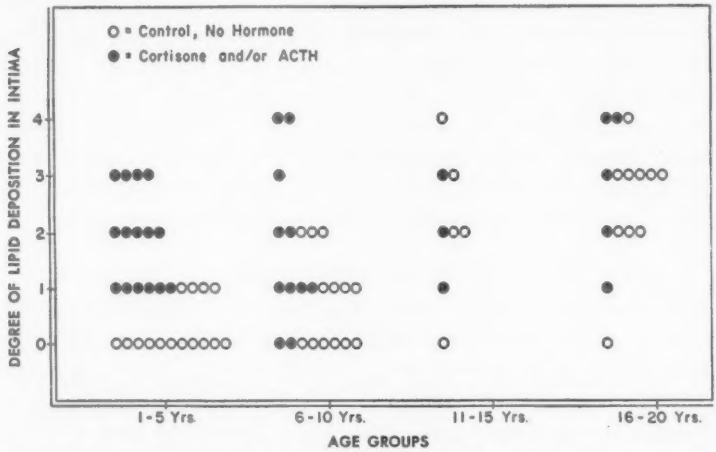
the controls that comparison between the two groups is equivocal. The number of cases over 11 years of age treated with cortisone was small and their treatment was brief.

In the media, material staining with Sudan IV was found. It was intracellular and arranged mostly around the nuclei. It consisted of distinct granules and never replaced the whole cell. Some similar ma-



Text-figure 1. Lipid in aortic intima. Comparison of patients treated with cortisone and/or ACTH and with no hormone.

terial was present also between the elastic fibers. Medial fat deposition was observed in 12 of 26 treated cases and in 2 of 28 controls in the 1 to 10 year age group (Table I). In the 11 to 20 year age group it was found in 3 of the 8 treated cases and in 1 of the 15 controls. Fourteen of the 15 treated patients and 2 of the 3 controls with this finding died of leukemia. Ten leukemic patients who had not received cortisone or ACTH did not have medial lipid.

The sudanophilic material gave a positive test for sterols using the Liebermann-Burchard reaction (Schultz) and it was precipitated with digitonin.[9] Only a part of the sudanophilic material, however, consisted of cholesterol or other sterols. These sterols seemed to be localized in the deeper layers of the intima. The fat-staining material in the media also gave a positive Liebermann-Burchard reaction.

With the hematoxylin and eosin stain, thickening in the intimal layers was found frequently in the group of children treated with cortisone. In general, the pathologic findings with the hematoxylin and eosin stain coincided with those found with Sudan IV. In a number of cases the hematoxylin and eosin stain showed intimal thickening in areas which did not stain with Sudan IV.

No difference was found between the treated and the control groups with Weigert's elastic tissue staining or periodic acid-Schiff's preparations of formalin-fixed tissues. In both groups polysaccharide-containing material was found between the elastic fibers with the latter method.

No definite correlation between the degree of change and the amount of hormone received could be established, but there seemed to be some

TABLE I

Lipid Material in Intima and Media of Children Treated with Cortisone or ACTH

Age group	Treatment	Total no. of cases	No. with leukemia	Cases with lipid in intima	Cases with lipid in media
1-10 yrs.	Cortisone and/or ACTH	26	24	24	12
	Control, no hormone	28	10	11 (3)*	2 (1)*
11-20 yrs.	Cortisone and/or ACTH	8	5	8 (2)*	3 (1)*
	Control, no hormone	15	2	13 (13)*	1

* Numbers in parentheses indicate number of patients with conditions other than leukemia.

relation to the duration of treatment. No definite difference in lipid deposition in the intima between cases treated with cortisone and those which received ACTH was found.

We are aware of the difficulties of interpreting findings in human post-mortem material. The time elapsed between death and necropsy varied, as did also the localization of the piece of aorta investigated. Moreover, many of the patients who were treated with hormone received additional treatment either with an anti-folic acid compound or with x-ray irradiation. The control aortas, however, were sampled similarly at random. The control leukemic patients were treated with anti-folic compound or with x-ray and they did not show lipid deposition in the aortas. It seems, therefore, that it is justified to assume that the changes in the aorta are related causatively to the administration of cortisone or ACTH.

SUMMARY

The aortas of 33 patients under 21 years of age who had been treated with cortisone and/or ACTH were compared with a suitable control group.

There appeared to be increased deposition of lipid in both the intima and media in patients under 11 years of age who received cortisone or ACTH. After age 11, lipid was found so frequently in the controls that comparison between the two groups is equivocal.

No correlation between the degree of change and the amount of

hormone received was found, but there seemed to be some relation to the duration of treatment.

There was no definite difference between the effects of cortisone and of ACTH.

REFERENCES

1. Adlersberg, D., Schaefer, L. E., and Dritch, R. Adrenal cortex and lipid metabolism: effects of cortisone and adrenocorticotropin (ACTH) on serum lipids in man. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 877–879.
2. Adlersberg, D., Schaefer, L. E., and Drachman, S. R. Development of hypercholesteremia during cortisone and ACTH therapy. *J. A. M. A.*, 1950, **144**, 909–914.
3. Adlersberg, D., Schaefer, L. E., and Drachman, S. R. Studies on hormonal control of serum lipid partition in man. *J. Clin. Endocrinol.*, 1951, **11**, 67–83.
4. Rich, A. R., Cochran, T. H., and McGoon, D. C. Marked lipemia resulting from the administration of cortisone. *Bull. Johns Hopkins Hosp.*, 1951, **88**, 101–109.
5. Hoffmeyer, J. Influence of adrenal cortex on cholesterol content in rabbit serum. *Acta physiol. Scandinav.*, 1945, **10**, 31–41.
6. Altschul, R. Selected Studies on Arteriosclerosis. Charles C Thomas, Springfield, Ill., 1950, 182 pp.
7. Schultz, A. Eine Methode des mikrochemischen Cholesterinnachweises am Gewebsschnitt. *Zentralbl. f. allg. Path. u. path. Anat.*, 1924–25, **35**, 314–317.
8. McManus, J. F. A. Histological and histochemical uses of periodic acid. *Stain Technol.*, 1948; **23**, 99–108.
9. Brunswik, H. Der mikrochemische Nachweis der Phytosterine und von Cholesterin als Digitonin-Steride. *Ztschr. f. wissensch. Mikr.*, 1922, **39**, 316–321.

DESCRIPTION OF PLATE

PLATE 44

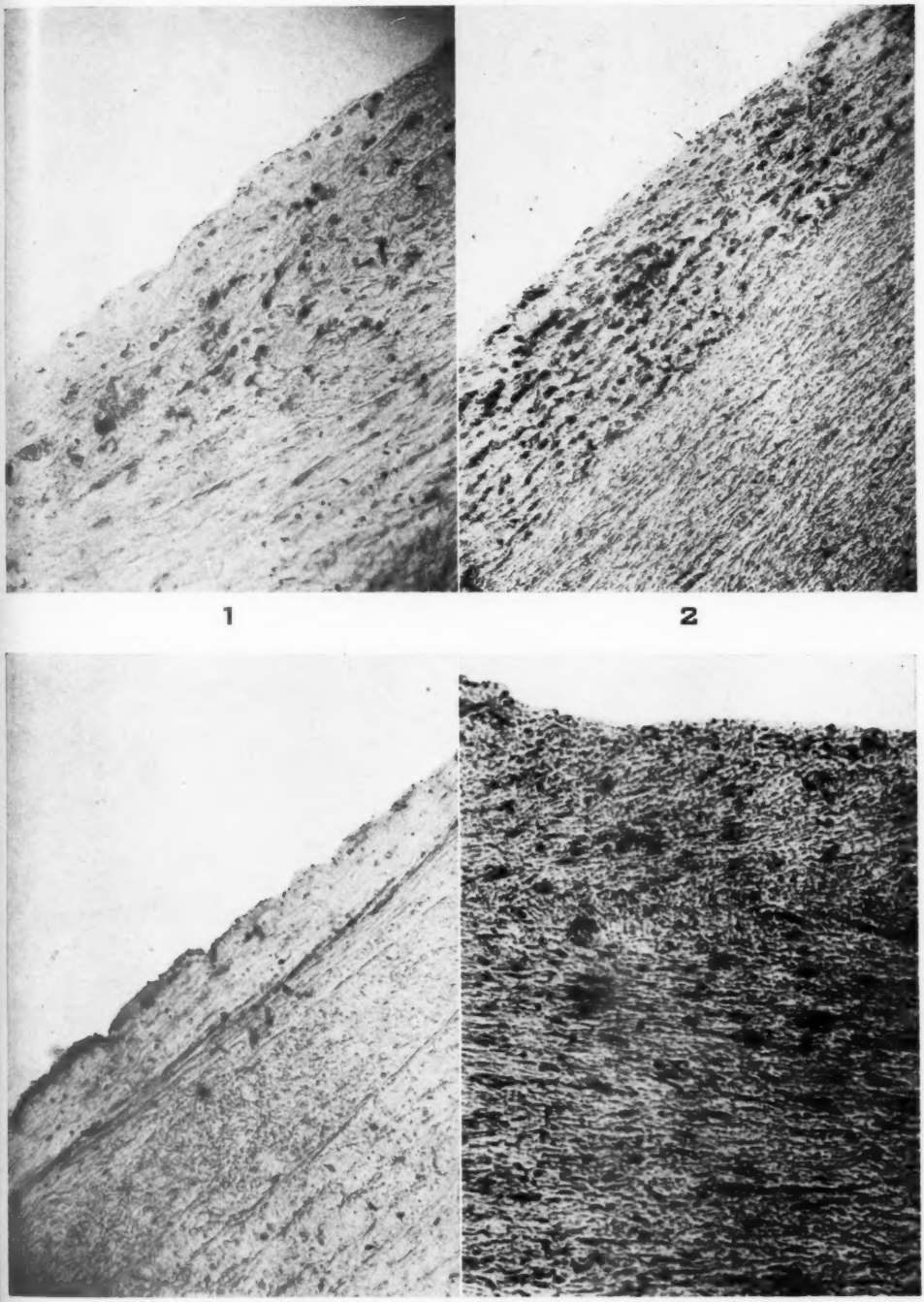
All photographs were made from Sudan IV preparations of the aortas of children treated with cortisone. The red areas of the Sudan stain are black in the reproduction.

FIG. 1. From the aorta of a boy, 3 years of age, who had acute blast-cell leukemia. He had received cortisone for 1 day, 50 mg., and ACTH for 12 weeks for a total of 2600 mg. × 240.

FIG. 2. The patient was an 8-year-old girl with acute lymphocytic leukemia. She had been given 7125 mg. of cortisone over a period of 24 weeks. × 120.

FIG. 3. A 14-year-old boy with acute myelogenous leukemia had been treated for 3 days with a total of 300 mg. of ACTH. Differential staining of the lamina propria is shown. × 240.

FIG. 4. A boy, 5 years of age, with acute stem cell leukemia had received 11,750 mg. of cortisone in 16 weeks. Lipid material appears in the media. × 240.



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3 4

Etheridge and Hoch-Ligeti Lipid Deposition in Aortas